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EXPERIMENTS ON LOCALIZATION IN THE EGGS OF A TELEOST FISH (FUNDULUS HETEROCLITUS)[1]

WARREN H. LEWIS

The Johns Hopkins University

TWENTY-NINE FIGURES

The ripe fish, Fundulus heteroclitus, are found in abundance at Woods Hole from the middle of June until the first week in August. The fish were stripped, the eggs immediately fertilized and kept in sea water at room temperature. About fifteen minutes after fertilization the germdisc becomes clearly visible although its limits are not sharply defined until from one hour and fifteen minutes to one hour and twenty-five minutes after fertilization. The first cleavage usually takes place about two hours after fertilization and the second cleavage plane appears about thirty minutes later. The rate at which cleavage takes place seems to vary with the temperature of the room.

The operations were very simple and were done under the binocular microscope. The eggs were held by a small pair of forceps with sufficient pressure to prevent the yolk from turning about within the vitelline membrane. With a very fine needle a puncture was made through the vitelline membrane into the germdisc or blastodisc at the point desired and as the needle was withdrawn a slight compression with the forceps would send out a stream of protoplasm from this region. The extent and position of the defect thus made in the egg can easily be seen through the vitelline membrane.

The changes following the operation, namely a rapid closing together of the tissue surrounding the defect, are so rapid that

[1] These experiments were done at the Marine Biological Laboratory, Woods Hole, Mass.

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only freehand outlines of the extent of the protoplasm or tissue removed could be made, and such were made for each experiment. In many of the eggs more or less of the yolk escaped soon after the operation and these were for the most part discarded. In some of the others the yolk escaped slowly but to such an extent as to cause death of the egg. The eggs which had been operated upon were kept in small covered glass dishes at ordinary room temperature.

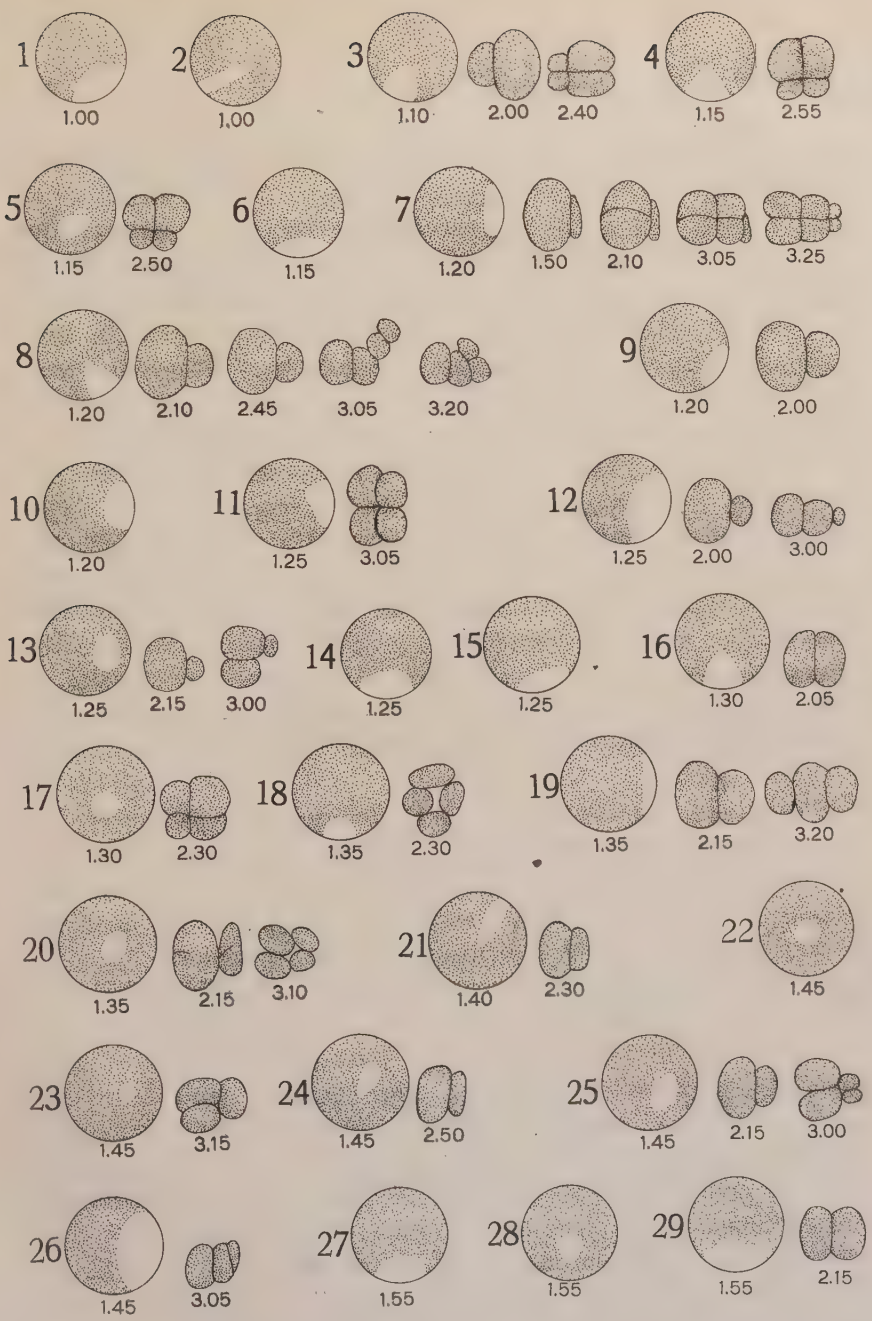
Experiments on the germdisc or one-celled stage

In order to determine whether there is any fixed localization of so-called organ forming substances in the germdisc, seventy-five eggs were operated upon at intervals from one to two hours after fertilization and various portions of the germdisc removed. Forty-six of the eggs died, many without cleavage, owing to the removal of too much of the germdisc or to the slow escape of the fluid yolk through injury to the yolk membrane, and in some the germinal vesicle was probably injured or removed.

Twenty-two eggs lived for a short time, eighteen until the formation of the blastodisc and four to the formation of the germ ring and early embryonic shield. The death of those in the blastodisc stage was often accompanied by the formation of a large vacuole beneath it, very similar to the conditions found by Stockard in certain lithium experiments.

Twenty-nine eggs developed into normal embryos as regards form and the relative proportions of the various parts. Many of them were smaller than normal. The extent and position of the protoplasm removed in these eggs is indicated in figures one to twenty-nine.

In all of the eggs, including those that failed to develop, the germdisc very quickly (one to three minutes) becomes rounded into a smaller disc, after the removal of a part of the protoplasm. Before the operation the germ disc appears homogenous except for a small central area and consequently no optical means are at hand to determine either a bilateral arrangement or the presence of organ forming substances.



Figs. 1 to 29 Diagrams of the germdiscs showing by the unstippeled areas the extent of the extirpated cytoplasm. Beneath each germdisc is the time in hours and minutes that elapsed between fertilization and the operation, and likewise beneath each cleavage stage is the time between fertilization and the observation of the stage drawn.

Figs. 1 to 29 indicate the amounts of protoplasm that were removed in each of the twenty-nine eggs. These areas when placed side by side cover an area between four and five times that of a single germdisc. It seems unlikely that any fixed areas of organ forming substances could have escaped being extirpated, and since all the embryos that developed from these eggs were normal except for size, we must conclude that either the cytoplasm of the germdisc is totipotent or that there is complete regeneration. There is no evidence of a mosaic such as exists in the Ascidian egg. It might be urged however that these experiments are not conclusive; in the first place, since such large numbers died, it might be that among these were the very ones from which the specialized areas were removed. In the second place a localization in layers parallel to the surface of the disc could exist, in which case removal of areas such as shown in the figures would only take a little from each and consequently no defects except in size would appear in the embryo. Experiments on later stages, however, indicate absence of localization as will be seen below.

Small but otherwise normal embryos develop from eggs that are operated upon at various periods from one hour and to one hour and fifty-five minutes after fertilization. There is no indication of any difference in the viability during this period nor of any increase in the differentiation of the cytoplasm.

In spite of the fact that there seems to be no differentiation of the cytoplasm there seems to be a very definite fixation of the position of the first cleavage plane. We have already pointed out that the germdisc quickly becomes rounded again after the removal of some of the protoplasm. The new disc is somewhat smaller than the original one. In spite of this it often happens that the first cleavage plane divides the germdisc into two unequal blastomeres. This inequality occurs even in eggs that were operated upon one hour and ten minutes after fertilization and at various periods between this and one hour and fifty-five minutes after fertilization. As shown in the figures there are varying degrees of this inequality between the first two blastomeres, a very great difference as in experiments 12 and 13 or a slight differ-

ence as in experiments 16 and 29. In 12 and 13 the extirpated cytoplasm probably came almost entirely from one only of the prospective blastomeres while in 16 and 29 it very likely came partly from each of the prospective blastomeres. In the figures the germdiscs are so arranged that the supposed potential first cleavage planes are vertical. If the first cleavage plane is fixed it becomes easy to explain the varying inequalities in the sizes of the blastomeres. Unless the first cleavage plane is already predetermined at the time of the operation we would expect the smaller rounded disc to divide into two equal blastomeres. It seems scarcely possible that the cytoplasm is a determining factor since it shifts so readily and flows into the extirpated area from the surrounding edge, yet how is it possible otherwise to account for the inequalities of the first two blastomeres. If the nucleus alone were the determining factor we would expect the smaller rounded disc to be equally divided. If we accept the view that the first cleavage plane is determined in the cytoplasm it is necessary to assume that a portion of it is more firm, reticular-like perhaps, than the more fluid part which flows in to fill up the wound.

The two-celled stage

Either one of the first two blastomeres may be removed and the remaining one will develop into a perfectly normal but smaller embryo. Morgan[2] succeeded in producing such embryos and I have also been able to do so without much difficulty.

The four-celled stage

More important however as regards localization are the operations on the four-celled stage. Any one of the first four cells can be removed and the remaining three will form a normal but slightly smaller embryo. Normal embryos will likewise develop after the removal of two of the first four cells, either the two daughter cells of one of the first blastomeres or two cells, one from

[2] Experimental studies on teleost eggs. Anat. Anz., vii, 1893.

each of the first two blastomeres. The formation of an embryo after the removal of any two of the first four blastomeres (except a diagonal couple) would indicate that there was neither an anterior posterior nor a bilateral arrangement of organ forming substances. Attempts to obtain embryos after the removal of three of the first four cells failed, probably owing to the small amount of embryonic tissue left behind.

The blastodisc

Many experiments on the blastodisc of eggs from five to twenty-four hours old, namely, from the sixteen or thirty-two celled stage until about the time when the germ ring first begins to appear show that here also there is no definite localization. Small portions (one-seventh to one-fifth) of the blastodisc were removed including various parts of the periphery and center. As in the one-celled stage the disc soon becomes rounded and development proceeds in a normal manner to the formation of a normal embryo. As in the preceding experiments injury to the yolk or removal of too great an area usually resulted in death. Otherwise the removal of small areas does not seem to affect the formation of a normal but smaller embryo.

CONCLUSIONS

Various portions of the germdisc or of the blastodisc of the teleost egg (Fundulus heteroclitus) can be removed yet normal but smaller embryos will develop and hatch, indicating that there is no definite fixed localization of organ forming substances.

When portions of the germdisc are removed the first cleavage plane often divides the disc into two unequal blastomeres, indicating that the position of the first cleavage plane is predetermined.

THE CULTIVATION OF SYMPATHETIC NERVES FROM THE INTESTINE OF CHICK EMBRYOS IN SALINE SOLUTIONS

WARREN H. LEWIS AND MARGARET REED LEWIS
From Johns Hopkins University[1]

TWENTY-SEVEN FIGURES

INTRODUCTION

The cultivation of embryonic nerve fibers from cells of the neural tube of amphibian embryos in clotted lymph by Harrison[2] and the somewhat similar results by Burrows[3] on the cultivation of nerve fibers from the neural tubes of chick embryos in clotted plasma would seem to place the out-growth theory of the origin of the nerve fiber upon a sound and impregnable basis. Such is not, however, the case in the minds of some of our contemporaries across the Atlantic.

Harrison was able to follow the outgrowth of long filamentous processes from cells of the neural tube, which had been transferred to a hanging drop culture. He could actually watch from minute to minute and hour to hour the lengthening of the primitive fibers and the continual changes in the amoeboid tips of the growing processes. He was able to study them with a D* Zeiss water immersion objective and a number 4 ocular, a magnification of about 400 diameters, in a few cases only could he use the higher oil immersion. The longest fiber observed was 1.15 mm. in length. They grew at rates varying from 15.6 μ to 56 μ per hour.

Burrows was able not only to follow the outgrowth of primitive nerve fibers in hanging drop cultures but succeeded in making permanent preparations with the Cajal reduced silver method and

[1] Through the kindness of Professor Kingsley we were able to continue the work at the Harpswell Laboratory.

[2] Anat. Record, vol. 1, 1907; Jour. Exp. Zool., vol. 9, 1911.

[3] Jour of Exp. Zool., vol. 9, 1911.

Held's molybdic haematoxylin as well as the more common stains. He was able to demonstrate the individual neurofibrillae in the growing nerve ends, and to prove beyond the possibility of a doubt that he was observing the growing nerve fibers.

Harrison and Burrows have demonstrated then that embryonic nerve cells will send out nerve fibers into clots of lymph and blood plasma. We[4] have demonstrated that nerve fibers grow readily in hanging drop cultures of pure saline solutions. The theory of Hensen that nerve fibers are formed by the differentiation of primitive protoplasmic bridges extending from cell to cell and that of Held's modification, that such bridges are organized from the cell peripheralwards through the influence of the nerve cell are both directly opposed to the experimental evidence referred to above.

The refusal to accept the evidence of experimental work is entirely unwarranted for only by such methods can we really analyze the processes which take place within the organism. In the face of Harrison's experimental evidence, which was published in full since the appearance of the papers by Hensen and Held we have the recent acceptance by Erick Müller[5] of the Hensen conception of the origin of the peripheral nervous system. Müller's evidence like that of Held is based upon histological studies from sections. The sooner anatomists come to realize the limitations of the histological method and come to utilize more and more the experimental method the better for the science of anatomy.

METHOD

The same method as described in our paper[6] on the cultivation of tissues from chick embryos in solutions of NaCl, $CaCl_2$, KCl, and $NaHCO_3$ was employed. A small piece of the intestine three or four mm. in length was put into a Petri dish containing about 10 cc. of the sterilized solution to be used in the hanging drop. The piece of intestine was then cut up into a number

[4] Lewis and Lewis, Anat. Record, 1911.
[5] Anat. Hefte, Bd. 43, 1911.
[6] Anat. Rec. 1911, p. 277.

of smaller pieces a fraction of a millimeter in diameter. These pieces were thoroughly washed in the solution, sometimes for nearly an hour, before being taken up one or two at a time in a fine capillary tube with some of the fluid to be transplanted onto the coverslip in a drop of the fluid thus taken up in the capillary tube. The coverslip was immediately inverted over a hollow ground slide on which had been placed a ring of stiff vaseline for the purpose of sealing the coverslip onto the slide to prevent evaporation. The slides were kept in a small thermostat at about 40° C. The Petri dishes and solutions were kept as a rule at about 35° to 40° C. during the manipulations, but this is not absolutely necessary. The drop and small pieces of tissue in it, always cooled down to room temperature, either in the capillary tube or on the coverslip, before the transplanting could be completed and the slide put into the thermostat. We did not seem to have quite as good results when the solutions in the Petri dishes were allowed to cool, but their cooling does not prevent growth.

The embryos were dissected under aseptic conditions. The instruments, slides, coverslips, capillary tubes, and vaseline were sterilized in a Bunsen flame. The cultures were examined on an ordinary warm stage. Permanent preparations were made by fixing the coverslips in osmic acid vapor and staining by the various methods. We have used with success Heidenhain's iron haematoxylin, long method, for the neurofibrillae and all the drawings from the permanent preparations, showing the neurofibrillae, were stained in this manner. The technique is so simple that we have no doubt but that our results will be easily confirmed.

MATERIAL AND MEDIA

The sympathetic nerve fibers are found in about 10 per cent of the cultures of the intestine and when they do grow out they are usually present in large numbers, indicating probably that the ganglia scattered along the intestine are only occasionally cut. The chick embryos used were from six to twelve days old.

The nerve fibers have been observed growing in a number of different solutions but no attempt has been made either to find

an optimum solution or to test their growth in a systematic manner in the various possible solutions. They have been observed growing in the following solutions of distilled water with (1) NaCl, 1.35 per cent; $CaCl_2$, 0.05 per cent; $NaHCO_3$, 0.04 per cent; (2) Locke's solution (NaCl, 0.9 per cent; $CaCl_2$, 0.025 per cent; KCl, 0.024 per cent; $NaHCO_3$, 0.02 per cent) + 0.05 per cent to 2 per cent dextrose; (3) Locke's solution + 0.25 per cent dextrose + 0.02 per cent or 0.04 per cent urea, or + 20 per cent bouillon, or + traces of ferricoxide, or + trace of $MgPO^4$; (5) Locke's solution with from 0.5 per cent to 1.35 per cent NaCl + 0.5 per cent to 2 per cent dextrose. Locke's solution + 0.25 per cent dextrose was used for a large majority of the cultures.

Advantages of a salt solution medium over that of lymph or plasma

The outgrowing nerve fibers are readily recognized after one has become familiar with the general characteristics of the cultures and are easily identified, at least the coarser ones, in the living. In the clotted lymph and plasma cultures of Harrison and Burrows the outgrowing nerves seemed to utilize the fibrin threads as supports, the nerves lying at various depths, often so far from the coverslip that they could not be studied with the oil immersion. One great advantage which the cultivation in salt solution possesses over the method of Harrison and Burrows depends on the fact that the outgrowing nerve fibers creep along the under side of the coverslip and seem to stick closely to it, i.e. they are probably stereotropic as Harrison[7] suggests. Thus the fibers are for the most part all in one plane or focus and can be followed continuously even with the oil immersion without difficulty. Another advantage is that in the fixing and staining the fluid salt solutions are much simpler to deal with than the more complicated plasma or lymph clot with its fibrin threads which often take the stain and obscure the picture presented by the growing tissues. The most important advantage however consists in the fact that we are dealing with solutions of known

[7] Science, Sept. 1, 1911.

chemical constitution and that our picture is uncomplicated by structures except those which have grown out from the original piece. This becomes extremely important when we study the outgrowing nerve fibers with their multitudes of fine branches that are often at the limits of visibility and seem to extend beyond even when observed with the 2 mm. oil immersion and the no. 12 comp. ocular. We can be quite confident that these fine fibillae depend for their origin on the nerves for no one would claim that solutions of NaCl, KCl, $CaCl_2$, $NaHCO_3$ with a little dextrose are capable of forming such fibrillae *de novo*.

The cultures often show areas containing only growing nerve fibers with their very delicate processes while other areas show only connective tissue-like cells with their fine processes, which are often extremely long and delicate but distinguishable from the nerves; in other places both kinds of cells are present. On the other hand a most careful study of the very finest of prepared microtome sections does not enable one to decide with perfect certainty the origin or relations of such extremely delicate structures owing to the very complex picture all sections of embryos exhibit from the intermingling of a variety of structures in a very intimate relationship, and of the formation of reticular like structures through coagulation of the intercellular fluids. The limitations of the study of microscopic sections must be recognized and supplemented by other methods.

The living cultures

The growth of various kinds of cells begins a few hours after the culture is made, the nerves first appear in from ten to twenty hours. The nerve fibers begin as simple threads with brush like ends (fig. 1). They usually grow in clusters from one or more areas of the piece, gradually growing outward in an irregular manner. The rate of growth varies in different fibers and at different times from less than $\frac{1}{2}$ μ to more than 1 μ per minute. As growth proceeds and the fibers extend farther and farther, the pattern becomes more and more complicated through anastomoses between branches of the same and different fibers and

through the outgrowth of more and more fibers from the central piece. In cultures from twenty-four to forty-eight hours old there is formed a dense jungle of nerve fibers of various sizes. Many of the nerve fibers attain a length of from $\frac{3}{4}$ to 1.2 mm.

Even in cultures ten hours old we find sometimes that the nerve fibers have already extended out some distance from the central piece and that anastomoses are already beginning to take place. Fig. 1 shows such a condition; some of the fibers were over $\frac{1}{2}$ mm. in length. If we follow one or two of these simple fibers from hour to hour, we see that they gradually increase in length and that the branching becomes more and more complicated, for example, fibers *A* and *B*, fig. 1, have grown considerably in the interval of twelve hours between figs. 1 and 2; and fig. 3, drawn from the culture two hours later, namely, the twenty-four-hour old culture, shows a condition still more complicated. If however we examine at 1000 diameters instead of 300 the picture is still more complex; compare regions *C* and *D*, fig. 3, with fig. 4, showing a nerve ending and anastomoses between branches of *A* and *B*; *E*, fig. 3, with fig. 5; *F* with fig. 6; and *G* with figs. 7, 8 and 9. Not only on the growing tips do we find numerous fine branches but at times the entire stem is richly covered and they may pass from larger fiber to larger fiber. Some of the fine fibrillae keep changing from minute to minute and although the figures referred to above from the living specimens, were drawn with the camera lucida, the picture does not represent precisely the actual condition at any one minute of the entire field for before one end is drawn the other has changed. Most of the fine anastomoses seen in fig. 5 were formed during the period of about an hour. The changes in the growing nerve tips are especially noticeable (see figs. 7, 8 and 9). The growing nerve tips are usually very rich in fine branches even when the more proximal portions of the fibers have but few of them, often in the older cultures the fine fibrillar branches disappear entirely from the main trunk of the nerve.

The nerve fibers as we have noted, usually begin to grow outward from the central transplanted piece ten to twenty hours after

the culture is made and continue to grow for twenty-four to forty-eight hours or occasionally even longer.

Harrison has pointed out that the main nerve paths in the embryo are probably determined by the configuration of the various organs and tissues and this together with the initial direction of the outgrowth from the neuroblasts and their protoplasmic activity suffices to bring the large nerve stems into the immediate neighborhood of the end organs or cells but just how the final gap is bridged between the larger nerve stem and the end organ or cells is not at all clear. The sending out of numerous branches in various directions by the growing nerve fiber offers a partial explanation as to the method by which the nerve fiber or its branches reaches the end organs in the normally developing embryo. The probabilities are that the growing nerve fibers, under the more favorable conditions of the embryo, are even more richly supplied with branches and much longer ones, than those seen in the artificial media. Again, not only the growing nerve fibers but the fine branches as well, seem to be stereotropic and are probably continually running out into the tissues using the mesenchymal cells and their processes as well as other more solid structures as supports and guides. The chances are, even though there are no definite paths or chemotactic stimuli from a distance, that every cell within a certain radius of the main nerve stem will be reached sooner or later by one or the other of the numerous branches which are being continually sent out and withdrawn only to be sent out in a new place. When such a branch finally reaches a cell of a certain type, a muscle cell for example, which is fitted by its constitution to be the end organ of a motor nerve, we can easily imagine a response of some kind resulting from the contact that will stimulate the activity of the nerve branch in such a manner as to cause it to retain its contact with the end organ and to grow in size. Branches coming in contact with cells of other types for which there is no such contact affinity are probably withdrawn again into the main stem in a manner similar to the reaction in artificial media where branches are continuously extended and withdrawn.

The presence of such fine branches running along the mesenchymal cells and their processes within the embryo might in fixed specimens easily lead to either the Hensen theory or the cell-chain theory of the origin of the peripheral nerves and but emphasises the advantages of the experimental method, in order to make clear the meaning of such confusing pictures.

The study of permanent preparations

The results in this paper based on the study of permanent preparations are from cultures killed in osmic acid vapor and stained with Heidenhain's iron haematoxylin. Figs. 12 and 13 give a fairly correct idea of the relations between the living and the permanent specimens. Fig. 12 shows the fine lateral anastomosing fibrillae running between four nerves arising from such different places on the central piece that they are in all probability from different cells. At the time this drawing was made we were not at all sure that all of the branches actually present were found as careful focusing continually revealed new ones. In the fixed preparation (fig. 13) the same field shown in fig. 12 was found without difficulty. The larger nerve fibers show shrinkage as does also the mesenchymal cell. Some of the fine fibrillae which were seen in the living specimen are broken (*A*) in the stained specimen. The latter reveals a few new fibrillae not seen in the fresh culture. But on the whole the two figures, one of the living and the other of the fixed preparation, are practically identical.

Some idea of the complicated networks the outgrowing nerve fibers produce may be gathered from figs. 14 and 15 drawn from fixed specimens of forty-eight and forty-two hour old cultures. The plexuses arising from the pieces are much more extensive than the portions shown in the figures. Fig. 14 shows but a small portion of the nerve plexus and includes a large nerve composed of numerous fine neurofibrillae. This fiber evidently arises from several neuroblasts within the piece. Its entire length is shown in the figure. There is also a rich anastomosing plexus of fine fibers many apparently consisting of but a single neurofibril. There are three mesenchymal cells entangled in the fibers which

might readily be mistaken for nerve cells. There were a few mesenchymal cells scattered among the nerve fibers of the plexus shown in fig. 15 but they have been omitted in the drawing.

There are indications here and there that the nerve fibers grow not only at the ends but likewise along their entire length as well. The spreading out which takes place at the crossing of two fibers as shown at *A*, fig. 19, and likewise the parallel course of two small fibers in the region where they cross obliquely as at *D*, fig. 18, are explainable through such growth along the fiber if we imagine adhesion between the two fibers at the point of crossing. We find also that the segments between branches of the living fibers show lengthening.

The neurofibrillae

The nerve fibers vary greatly in diameter, some of the fine ones, as *B*, *C*, *E*, fig. 19, *A*, fig. 18, and *A*, *B*, fig. 16, seem to consist of but a single neurofibril. The fiber *C*, fig. 16, splits into two neurofibrillae as it crosses over the two large mesenchymal cells; one is unable, however, to distinguish the two fibrils at *C*. The fiber *F*, fig. 19, splits into three neurofibrillae as it crosses the larger nerve at *A*, but one is unable at *F* to distinguish the three fibrils. The larger nerves as shown in figs. 14, 16, and 19 consist of many neurofibrils. The picture varies with the depth of the stain. In the lighter stained specimens, fig. 14, one can distinguish very readily the many neurofibrillae in the larger nerve. In the more deeply stained specimens however the neurofibrillae are very difficult to distinguish except in places where the nerves are flattened out or drawn apart (see figs. 16 and *A*, 19) either in crossing each other or in crossing over a mesenchymal cell. The neurofibrillae seem to be more apparent in the proximal portions of the nerves than towards the nerve endings. The fiber in the neighborhood of the nerve ending may have a greater diameter than a more proximal fiber showing several neurofibrillae yet show no signs of fibrillae even when the fiber is flattened out in passing over a mesenchymal cell as in fig. 17.

It is rather puzzling at times to determine whether the lateral anastomosing branches of the nerve endings as *C*, *C*, *C*, fig. 18

are to be considered as neurofibrillae or merely fine branches of the more primitive nerve fiber. It appears in places as if such fine branches may persist and become converted into single neurofibrillae.

In favorable specimens the neurofibrillae are seen to be granular in structure (figs. 16 and 19). Not only the neurofibrillae of the larger nerves but the isolated neurofibrillae as well seem to consist of *neurogranules*. In some specimens all of the neurofibrillae are granular while in others the extraction of the stain has not gone far enough and we get a solid black appearance in which the larger nerves show neither the neurofibrillae nor the neurogranules. If the extraction is carried too far the granules seem to disappear. The reaction here is strikingly similar to that of the chromosomes of the nucleus.

The nodosities so characteristic of the sympathetic nerve fibers, abound everywhere, most pronounced perhaps on the fibers leading to the nerve endings (figs. 21, 23, 26). They are also common on anastomosing lateral branches in the region of the nerve endings. Not only do they occur on the more primitive portions of the nerve fibers, where neurofibrillation has not developed, but they are likewise found on the individual neurofibrillae (*A*, fig. 18, *B*, *D*, *G*, fig. 19. They very frequently occur at the junction of two nerves *B*, *D*, fig. 19). There seems to be no regularity in the method of distribution. In favorable places when the stain is just right they are seen to be granular in structure, with *neurogranules* similar to those in the nerves and neurofibrillae. When the stain is deep and the fibers and neurofibrillae are homogenous black they are likewise solid black. They apparently consist of the same material as do the nerve fibers.

We are not prepared at present to decide with very much certainty as to the real significance of the *neurogranules*. They may in part be merely due to peculiarities of the stain or method of preparation.

The nerve endings

The fixed specimens show even better than the living ones the characteristic amoeboid nerve endings (figs. 20 to 27). They vary greatly in size and appearance, often consisting of a finely gran-

ular membrane-like portion with numerous very fine branches which usually appear to run beyond the limits of visibility even with the 2 mm. oil immersion and compensating No. 12 ocular (Zeiss). In the living cultures, as well as in the fixed specimens we find that some of the fibers leading to the endings are devoid of the fine lateral branches (fig. 23) while others are richly supplied (fig. 25). Anastomoses with neighboring nerve endings and fibers are frequent (*C*, *C*, *C*, fig. 18, figs. 25, 26, and 27). Not only is the nerve ending itself granular but the nerve fiber leading to it seems likewise to be granular (*A*, fig. 21). These granules react to the stain like those found in the neurofibrillae.

CONCLUSIONS

Small pieces of the intestine from embryonic chicks often send off sympathetic nerve fibers when cultivated in various saline solutions.

Such nerve fibers develop rapidly in these solutions and may attain a length of over one mm.

They show the characteristic amoeboid nerve ends and are rich in lateral branches and true anastomoses with neighboring fibers.

The fibers creep along on the under side of the coverslip; they probably are stereotropic.

The fixed preparations stained with Heidenhain's iron haematoxylin show the neurofibrillae, the nodosities, the nerve endings and the primitive nerve fibers. They appear to be granular in structure. These *neurogranules* react to the iron haematoxylin very much as do the chromosomes.

The sympathetic nerve fibers like those from the central nervous system are outgrowths from nerve cells and are not formed from preëxisting protoplasmic networks.

PLATE 1

EXPLANATION OF FIGURES

The figures are all reduced one-half from camera drawings.

Figs. 1 to 9 Sympathetic nerve fibers from a living culture of the intestine of a ten-day chick embryo. Culture media: NaCl, 0.5 per cent; $CaCl_2$, 0.025 per cent; KCl, 0.042 per cent; $NaHCO_3$, 0.02 per cent, and dextrose 0.5 per cent.

1 Nerve fibers in ten-hour old culture. × 150 dia.

2 Nerve fibers *A* and *B* of fig. 1, twelve hours later. × 150 dia.

3 Same fibers two hours later, namely, twenty-four hours after the culture was made. × 150 dia.

4 Nerve ending at *C*, fig. 3, in the twenty-six-hour old culture. × 500 dia.

5 Enlargement of region *E*, fig. 3, in the twenty-five-hour old culture. × 500 dia.

6 Enlargement of region *F*, fig. 3, twenty-six-hour culture. × 500 dia.

7–9 Nerve ending at *G*, fig. 3, in twenty-four, twenty-four and one-half and twenty-five-hour cultures. × 500 dia.

1
A
B
4
D
2
A
B
G
7
8
9
E
D
C
F
6
5
3
A
B

PLATE 2

EXPLANATION OF FIGURES

10-11 Sympathetic nerve endings from a twenty-six-hour old living culture of the intestine of an eight-day chick embryo in Locke's solution (NaCl, 0.9 per cent; $CaCl_2$, 0.925 per cent; KCl, 0.042 per cent; $NaHCO_3$, 0.2 per cent) + 0.25 per cent dextrose. × 500 dia.

12 Sympathetic nerve fibers from a twenty-eight-hour old living culture of the small piece of the intestine of an eight-day chick embryo in Locke's solution + 0.25 per cent dextrose. The drawing shows four separate fibers (*F, O, U, R.*) with many fine connecting branches. × 500 dia.

13 Same specimen as above after fixation and staining. × 500 dia.

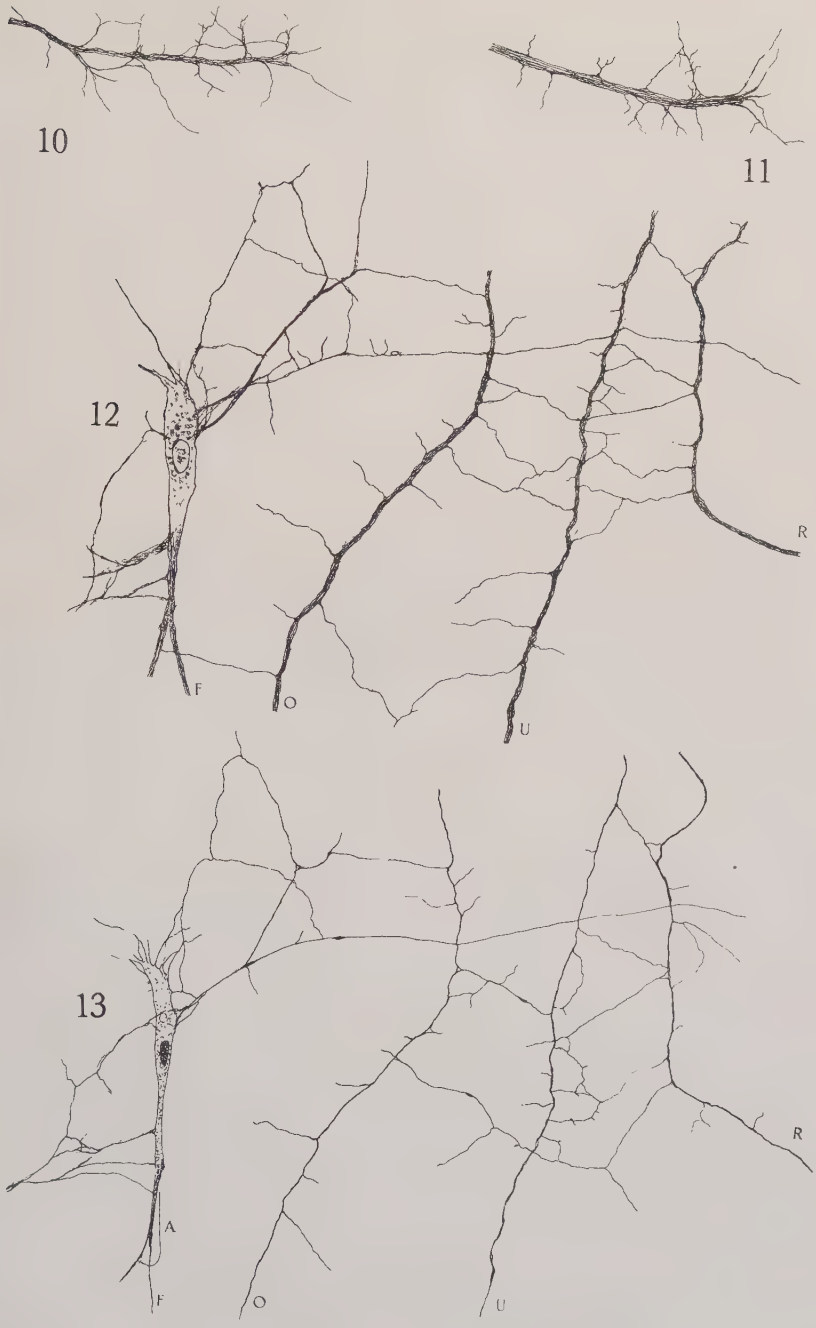

10
11
12
F
O
U
R
13
A
F
O
U
R

PLATE 3

EXPLANATION OF FIGURE

14 Portion of a sympathetic nerve plexus from a forty-two-hour old culture of a small piece of the intestine of a six-day chick embryo in Locke's solution + 0.25 per cent dextrose. *H*, *H*, *H*, mesenchymal cells. × 750 dia.

14
H
H
H

PLATE 4

EXPLANATION OF FIGURES

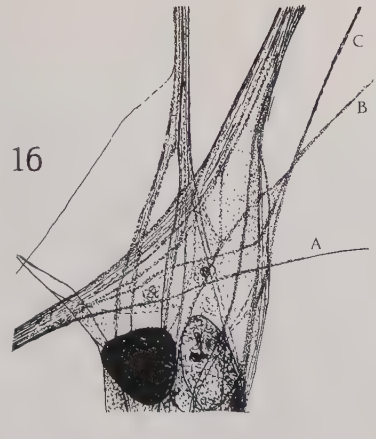
15 Sympathetic nerve plexus from a forty-eight-hour old culture of a small piece of the intestine of an eight-day chick embryo grown in modified Locke's solution, containing 1.35 per cent NaCl and instead of 0.9 per cent, + 0.25 per cent dextrose. × 150 dia.

16–24 From plexus shown in fig. 15. × 1500 dia. (Zeiss 2 mm. objective and 12 comp. ocular.)

16 Large nerve passing over group of mesenchymal cells spread apart showing neurofibrillae and *neurogranules*.

17 A much flattened primitive fiber passing over a mesenchymal cell.

15



16

17

PLATE 5

EXPLANATION OF FIGURES

18 Plexus with three nerve endings *C*, *C*, *C*. × 1500 dia.

19 Nerve fibers flattened out at point of crossing *A* revealing neurofibrillae and *neurogranules*. Some of the individual neurofibrillae show granules as at *C*. × 1500 dia.

18

19

PLATE 6

EXPLANATION OF FIGURES

20–24 Nerve endings. × 1500 dia.

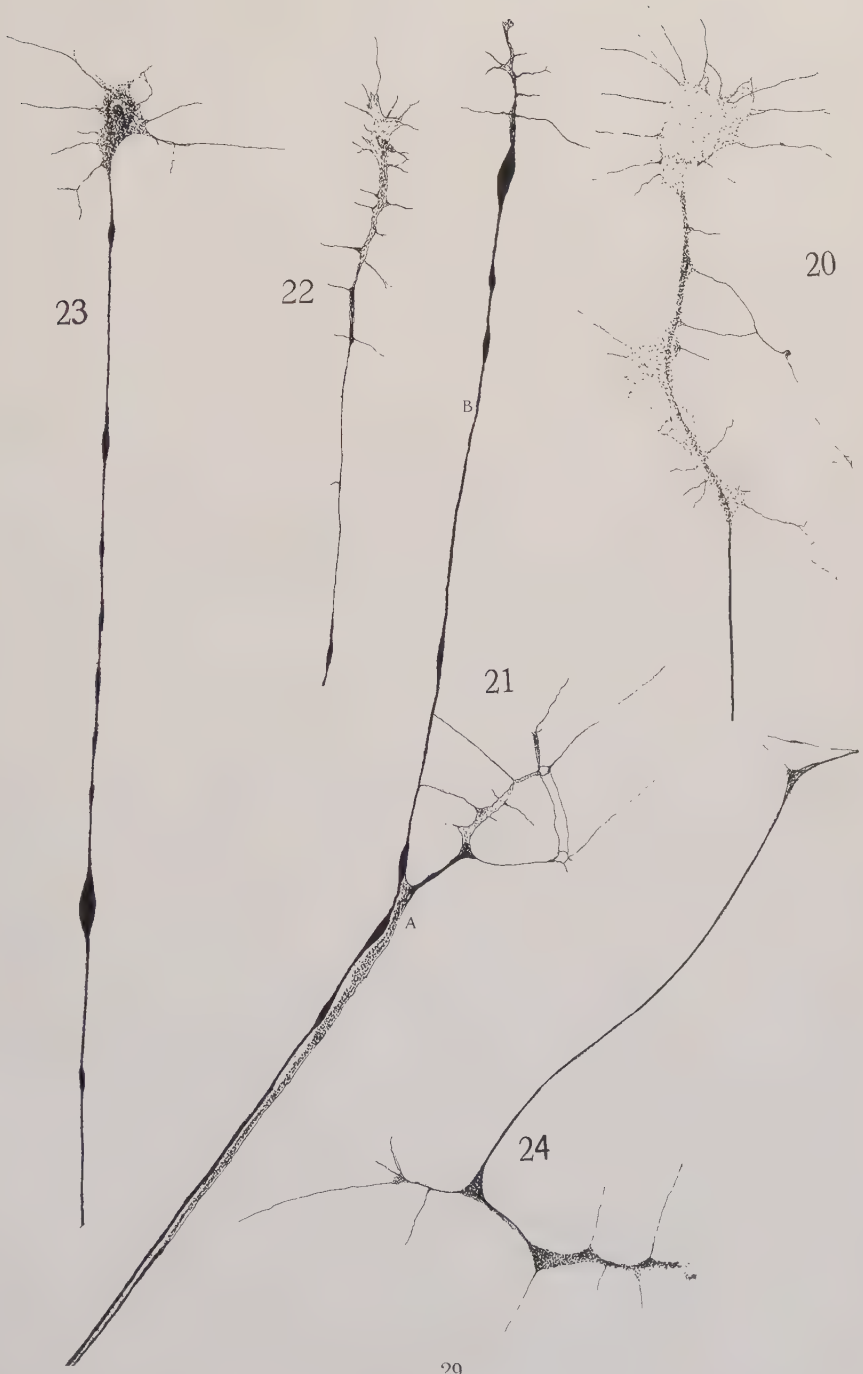
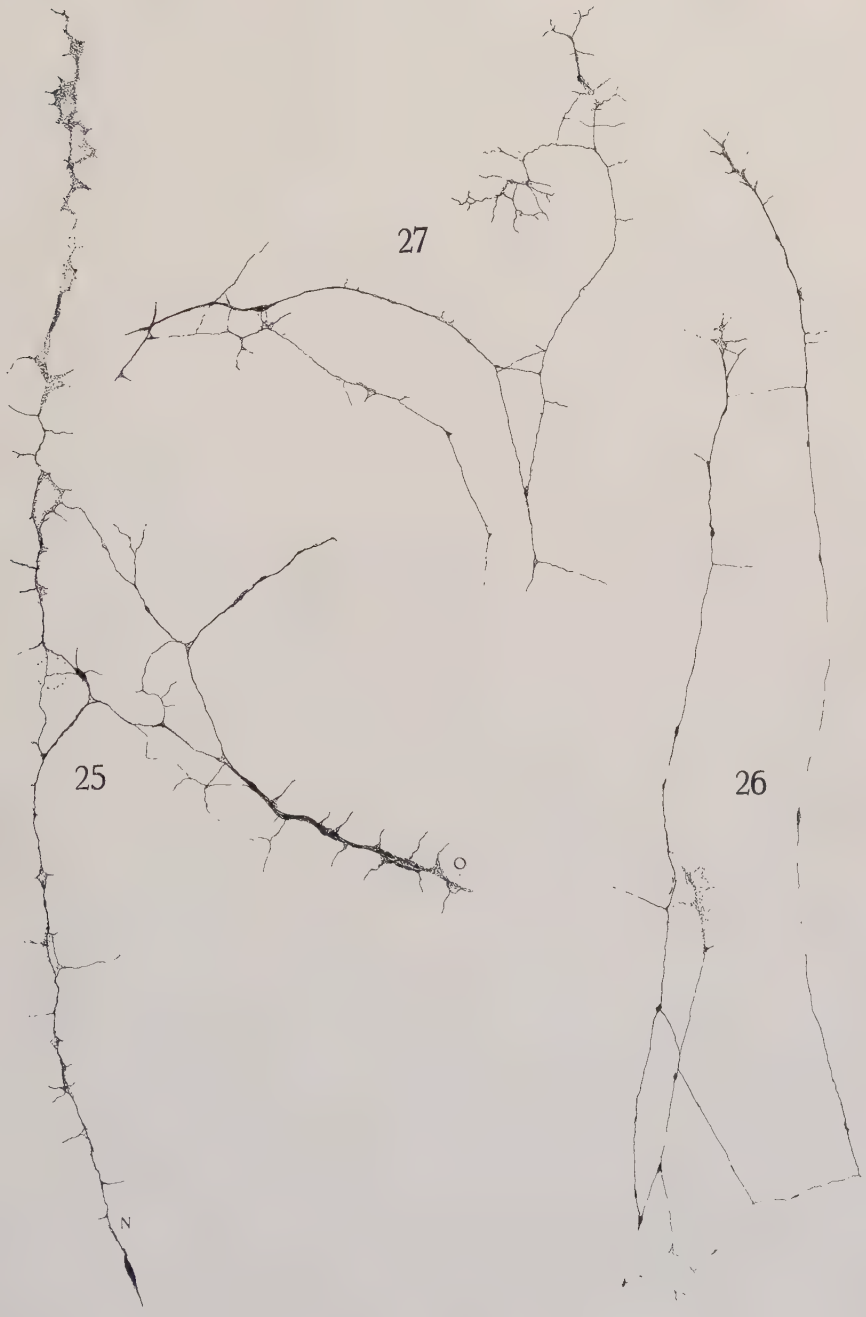

23
22
20
B
21
A
24

PLATE 7

EXPLANATION OF FIGURES

25 Nerve endings from forty-two-hour culture of an eight-day chick grown in modified Locke's solution with only 0.5 per cent NaCl and with 1.5 per cent dextrose added; *N*, *O*, branches from different nerves. × 550 dia.

26–27 Nerve endings from a forty-two-hour old culture of an eight-day chick grown in Locke's solution + 1.5 per cent dextrose. × 550 dia.


27
25
26
O
N

MITOCHONDRIA AND OTHER CYTOPLASMIC CONSTITUENTS OF THE SPINAL GANGLION CELLS OF THE PIGEON

PRELIMINARY NOTE

E. V. COWDRY

From the Anatomical Laboratory of the University of Chicago

Neurologists have been actively investigating the problem of the structure of nerve cells for many years. The amount of literature is enormous. This is partly due to the lack of correlation of the results which have been obtained. In spite of the large number of workers engaged, investigators were for a considerable period content to describe structures in the nerve cell without determining their relations to bodies previously discovered by others. As a result, numerous synonyms have accumulated, and, of late years, the most heated controversies have arisen from attempts on the part of different investigators to correlate their results with those of others without sacrificing their own claim to priority. This state of confusion is one of the factors which has deterred the general cytologists from entering a field in which so much has already been accomplished but where the very names of the bodies described are in so active debate. So that our knowledge of the structure of the nerve cell has developed in advance and almost independently of the progress which is being made in the study of the other cells of the body. This condition of affairs is, on the face of it, extremely illogical; for all cells are, in a sense, fundamentally similar: they are at least developed from the same germ cell. The nerve cell is not a structure apart, and different from all other cells. Even the great discoveries which are being made, day by day, by the general cytologists relating to mitochondria and other cytoplasmic constituents in other than nerve cells have not been effectually linked up with the structures to be

observed in the nerve cell. It was, therefore, with the hope of being able to aid in this correlation that this investigation was initiated. Before this can be done, however, the relations of the structures which have already been observed and described in the nerve cell to one another has to be established.

The general method of approach employed is that of synthesis, or, in other words, the simultaneous or successive demonstration in a single nerve cell of all of its known cytoplasmic constituents. This may be accomplished in three ways. (1) In rare instances by the application of a single method of technique, such as Bensley's acid fuchsin methyl green method. (2) By staining one of the components in a specific fashion, for example the canalicular apparatus with osmic acid, followed by other dyes to show the Nissl substance, the mitochondria, etc. (3) By staining one constituent, observing it, and then decolorizing and restaining the other constituents in the same cell by appropriate methods. Spinal ganglion cells of the pigeon were chosen as material since the ganglia are easily obtained and are readily penetrated by fixing fluids.

The neurosomes of Held were taken as the point of departure. These granules often exhibit a tendency to be arranged in rows which radiate up into the cytoplasm from the axone. They have frequently been confused with neurofibrils. Thus, Martin Heidenhain ('11, p. 829) considers that the neurosomes are neurofibrils stained in an incomplete and discontinuous fashion. I made preparations by the three methods employed by Held for the demonstration of neurosomes, and the granules observed seemed to agree very closely with his descriptions and figures. Those seen in the erythrosin-methylene blue specimens (first method, '95, p. 399) could be sharply differentiated from the rod-shaped neurosomes which appeared in preparations prepared by Held's Altmann and iron hematoxylin methods ('97, p. 228, and '97a, p. 275) on the basis of the variable size and irregular shape of the former, and on account of the fact that they were densely crowded together in the axone hillock.

I was unable to determine the nature of these erythrosin-stained neurosomes. The fact that they occur and can be

readily stained after a large number of fixations, such as formalin, strong Flemming, formalin-Zenker, trichlorlactic acid, chrome sublimate, etc., is against the view that they are fixation artefacts. These fixatives are, however, strong precipitants and this evidence is therefore not very trustworthy. On the other hand, the uniformly rounded, rod-like neurosomes, observed in the Altmann and iron hematoxylin preparations, were positively identified as mitochondria, since their morphology and staining affinities were the same as mitochondria which were demonstrated in the same tissue by the following methods: (1) Michaelis ('99, p. 565) janus green intra vitam (see also Bensley, '11, p. 304). (2) The Benda method as employed by Meves and Duesberg ('07, p. 574). (3) The iron hematoxylin method of Heidenhain as applied by Meves ('08, p. 832). (4) Bensley's ('11, p. 310) copper chrome hematoxylin method. (5) Bensley's ('11, p. 309) acid fuchsin methyl green method. (6) Bensley's ('11, p. 309) neutral safranin method. (7) Kingsbury's ('11, p. 317) modification of the Weigert hematoxylin method.

The morphological independence of the mitochondria and the Nissl or chromidial substance was shown by the application of the technical methods just enumerated; for they were repeatedly observed side by side in the same cell differentially stained.

The canalicular apparatus was studied by the application of the following methods of technique: (1) Holmgren ('99, p. 389) fixation in picric acid sublimate, sublimate acetic and Carnoy's 6 : 3 : 1 fluid (canalicular apparatus as clear, uncolored spaces). (2) Nelis ('00, p. 613) fixation in 7 per cent formalin and staining in iron hematoxylin and Böhmer's hematoxylin (clear, colorless spaces). (3) Holmgren ('01, p. 297) fixation in trichlorlactic acid and staining in Weigert's resorcin-fuchsin (canalicular apparatus dark purple). (4) Kopsch ('02) 2 per cent osmic acid (black). (5) Bensley's ('10, p. 192) fixation in formalin bichromate sublimate mixture (clear, uncolored spaces). (6) Kingsbury's 1911 modification of the Weigert hematoxylin method (canals dark brownish black). (7) Canalicular apparatus appears as tortuous, colorless canals against a highly granular red stained background when treated with 1 : 1000 solution of pyronin in 0.75 per cent

sodium chloride solution intra vitam. The canalicular apparatus demonstrated by these methods was identified as a single component of the nerve cell since (1) the characteristic diffuse, excentric and circumnuclear types of formation could be generally distinguished; and (2) because, in the cases where it was positively stained, all grades could be observed, in a single section, between cells containing unstained canals, those exhibiting unstained and a few colored canals, and cells with the canalicular apparatus completely stained.

The occurrence of these different forms of canalicular apparatus presents a surprising degree of constancy, in view of the fact that we with Bensley conceive of these intracellular canals as an osmotic mechanism which varies in form from moment to moment in the living cell; for these three types have a fairly constant distribution. The diffuse form occupies almost the whole extent of the cytoplasm of the large ganglion cells; the excentric consists of a circumscribed network of canals placed in an asymmetrical position at one or other side of the nucleus, and occurs generally in the medium sized cells; while the circumnuclear type of canalicular apparatus forms a continuous anastomosing network which closely surrounds the nucleus and occurs, almost invariably, in the small spinal ganglion cells. Nevertheless infinite variation was observed in the diameter, shape and volume of the canals in neighboring cells in the same section.

This canalicular system is morphologically independent of the mitochondria and of the Nissl substance, with both of which it has been confused; for Kopsch preparations may be counterstained by Bensley's acid fuchsin toluidin blue method, if the treatment with potassium permanganate and oxalic acid be omitted. When this has been done the canalicular apparatus appears black, the mitochondria red and the Nissl substance dark blue.

Specimens were prepared by a modification of Cajal's silver impregnation method and were subsequently counterstained with safranin. In them deep blue-black neurofibrils could be seen and the canalicular apparatus stood out sharply as clear uncolored spaces winding in and out against a brightly red stained background. Flakes of Nissl substance were brought out between the

neurofibrils in specimens which were counterstained with toluidin blue in place of the safranin.

There are three well-known reasons in justification of the general conception that the Nissl substances belongs to the category of chromidial substances. In the first place it stains with basic dyes, such as toluidin blue, methylene blue, etc., after fixation in neutral fluids. Secondly it, like the chromidial substance, is formed as the result of nuclear activity. Furthermore, it appears to play a similar rôle in the metabolism of the nerve cell to that which the chromidial substance has been shown to do in other than nerve cells. It has been demonstrated above that mitochondria bear no morphological relationship to the Nissl substance. Since the Nissl substance and the chromidial substance in the nerve cell are identical it follows that confusion of the latter with mitochondria is unjustifiable.

Finally, mitochondria, chromidial substance, canalicular apparatus and neurofibrils may be demonstrated side by side in the same cell by the application of the acid fuchsin methyl green method above mentioned.

The conclusions resulting from this investigation are as follows:

1. Mitochondria occur in adult spinal ganglion cells of the pigeon, where they may be demonstrated by means of all the methods in use for their study in other than nerve cells.

2. The neurosomes of Held are not a single, separate and distinct form of cell granulation. They may be divided into two types. Type I consists of those which Held observed by the application of his erythrosin-methylene blue method, the nature of these is unknown; and type II is composed of the rod-shaped bodies which he studied in his Altmann and iron hematoxylin preparations. These type II neurosomes are mitochondria.

3. There are four fundamental known components in the cytoplasm of nerve cells, which, in the adult at least, are morphologically independent, and which are not transformed one into another, either by fixation or in any other way, namely: the mitochondria, the Nissl bodies, the canalicular apparatus and the neurofibrils. The mitochondria are the chondriosomes, chondriocontes, and chondriomites of Meves and others; the Nissl bodies

are frequently referred to as chromidial apparatus, tigroid substance, chromatophile substance, etc.; and the canalicular apparatus is identical with system of clear intracellular canals described by Von Bergen, Bensley and others, the spiremes of Nelis, the Binnennetz of Kopsch and the Saftkanälchen of Holmgren.

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Buffalo, N. Y., January 1, 1912.

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It is our highest ambition to build up a scientific manufacturing institution here in America, the members of which shall keep in close touch and sympathy with the laboratory workers of the country and go right into the problems which you have in mind with the idea of doing our part to solve them. To that end, we have worked slowly and carefully, using our ingenuity, profiting by our experience in practical laboratory work, designing and building our instruments from the laboratory workers standpoint, making sure that we accepted no more business than we could do and do right and ever showing a willingness, in fact a desire to fully co-operate in working out the problems entrusted to us. This will continue to be our policy.

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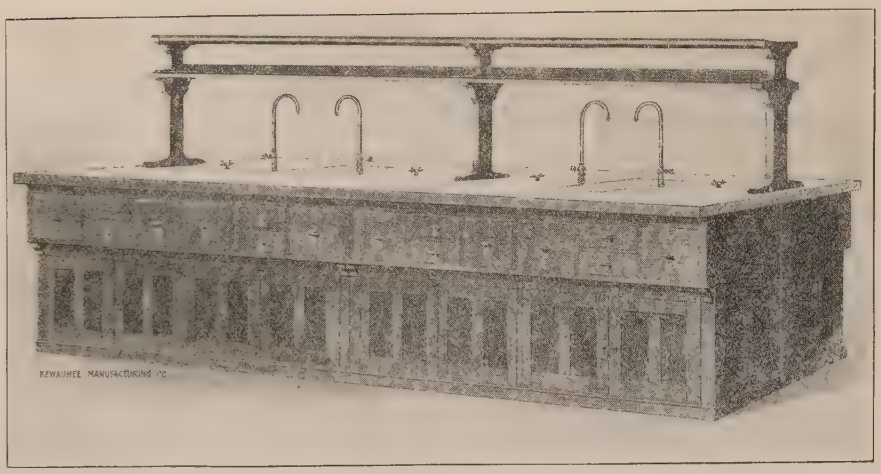

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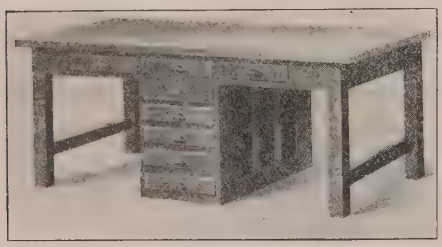

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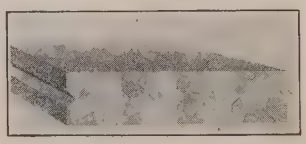

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CYTOPLASMIC FIXATION[1]

B. F. KINGSBURY

From the Department of Histology, Cornell University

Of the chemicals employed for the fixation of protoplasm, osmium tetroxid (osmic acid), potassium bichromate, and formaldehyde constitute a group by themselves (Fischer's group II) and possess several features in common. To them should be added chromium trioxid (chromic acid) which naturally belongs with the dichromates.

As is generally conceded, they are good preservatives of cytoplasm. This is attested by the large number of fixing fluids devised in which they are (essentially) the only important constituents—such as Altmann's, Benda's, Bensley's, Helly's, Kopsch's, Orth's, Regaud's fluids, and others—or solutions in which their presence contributes largely to the excellence of the result, such as Zenker's fluid, Tellyesniczky's fluid, etc.

In the case of the formulae of the first group (Altmann's, etc.) they were devised for the preservation of cytoplasmic granules of apparently diverse kinds, such as pepsinogen and trypsinogen granules as well as other kinds, including the mitochondria (chondriosomes). It is unnecessary at this point to furnish detailed illustration.

Not only do they afford a preservation of the more labile granules that occur in the cytoplasm, but they give an excellent preservation of the general form of the cytoplasm. Orth's fluid and Helly's fluid are particularly and deservedly popular as preservatives of such difficult cells as those of the renal tubules.

[1] Read by title at the Ithaca meeting of the Association of American Anatomists, December, 1910, and since extended by studies in the Histological-Embryological Institute, Munich. Of the generous help freely given by Professor Mollier and Dr. Boehm, I wish to express my sincere appreciation and thanks.

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FEBRUARY, 1912

Bensley ('10) has recently given in an interesting paper good evidence of their value in the maintenance of cytoplasmic morphology.

As fixers, they possess three rather striking peculiarities:

1. In contradistinction to the other chemicals employed as fixers they do not appear to cause a coagulation or precipitation of proteins. The peculiar gelatinous appearance formaldehyde gives to the tissues is well known and characteristic. In the case of bichromates and osmium tetroxid the browning and blackening that they occasion disguises any 'gelatinizing' effect they may have. In Mann's table ('03) it is interesting to note that the only protein substance that they precipitate is hemoglobin and that the remaining proteins were precipitated only in acid solutions. The precipitation of the hemoglobin in an insoluble form is perhaps to be considered as associated with an oxidation (vide subseq.). The effect of these chemicals on proteins however does not seem to be at all well understood. In any event they are similar in their action and belong in a group together, as Fischer ('99) put them.

2. They are preëminently oxidizers. In the case of osmium tetroxid and potassium bichromate this requires no special comment. Formaldehyde is however a reducing substance and at first thought would seem to be diametrically opposite to the first two in its effect. Chemists,[2] however, give information that in its relations to many organic substances, probably including proteins, formaldehyde may act essentially as an oxidizer, furnishing an atom of oxygen that combines with two atoms of hydrogen, the remainder of the formaldehyde molecule (CH_2) being incorporated with the 'reducing' molecule (methylenization). This is the view taken by Blum ('10). It is probably because of their oxidizing power that these chemicals possess a distinct value as preservers of fat. Compare Tellyesniczky ('10).

3. They are poor or indifferent preservatives of nuclear structure. Potassium bichromate is in fact a solvent of the nuclear structures, as was shown by Burckhardt ('97). In this respect,

[2] Dr. Hunter, professor of biochemistry, has in the course of numerous conversations given helpful information and suggestions.

as shown by him, bichromates of the different metals show marked differences, calcium bichromate and copper bichromate being much superior, even good, nuclear fixers. The homogeneous—structureless—appearance of nuclei fixed in simple potassium bichromate solution (e.g., Müller's fluid) is well known.

Osmium tetroxid likewise gives to the nucleus a characteristic homogeneous, glassy appearance in which detail of chromatin structure is lost. Rawitz ('07), Kotlarewsky ('87) and Eisen ('00) have emphasized its inability to preserve nuclear detail.

As far as formaldehyde is concerned, opinions of its value as a preserver of nuclear structure differ widely. It should be remembered in this connection however that formaldehyde is rarely employed pure, small amounts of formic acid being usually present. Certainly it is much superior to the other two chemicals under discussion, though still, in the opinion of the writer, an inferior preserver of nuclear structures.

As employed in the fixers of the first group (Altmann's, etc.) with special reference to the fixation of cytoplasm, acetic or other acid, which is so important a constituent of nuclear and general fixers is absent, or present only in minimal amount. This is particularly true when the preservation of cytoplasmic granules is desired, although, according to the recent study of Bensley ('10), the before mentioned acid must be omitted for the best preservation of the general morphology of the cell body. It is unnecessary to go into this at present; the studies of the numerous glandular organs that have been made for a number of years, of which those of Bensley and his students may be specially mentioned, have shown this well.

These chemicals, possessing such characteristics in common, are thus preëminently preservers of 'cytoplasmic granules' of a certain class. By means of their use Altmann built up his Granula Theory, which is being revived in modified form in the mitochondria work so popular today. That the granules of Altmann and the mitochondria were the same has been recognized by Meves ('10 a) and demonstrated by himself and by his pupil, Samssonow ('10) and the theory erected upon the framework of mitochondrial fact bids fair to be as pretentious as the earlier

one, though less extensive in its application. The mitochondria (chondriosomes, etc.) by transformation form the fibrillae of connective tissue (Meves, '10 b), the neurofibrillae in the growing neuroblast (Hoven, '10), the myofibrillae (Duesberg, '10), the fibrillae of epithelial cells (Favre, '10, Firket, '11), and play a part in the process of cornification (Firket '11). Directly or indirectly, they contribute the secretory granules in the salivary (Regaud and Mawas, '09, Bouin, '05), gastric (Schultze, '11), pancreatic (Hoven, '10; Schultze, '11), mammary (Hoven, '11) and other glands (e.g., Schultze, '11). They are described in the rods of the urinary tubule cells (Schultze, '11; Regaud, '08), intestinal cells (Champy, '10), Liver cells (Policard, '09). They are abundantly present in the oocyte and spermatocyte (Benda, '97; van der Stricht, '00; Meves, '11, and others) and in the spermatozoon are carried over into the egg cell in fertilization (Benda, Meves, '11). They abound in the cells of the young embryo (Meves, '08; Rubaschkin, '11); they occur in plant cells as well as animal (Duesberg, '10; Meves '04). It is but natural, therefore, that they should be regarded as important formative structures in protoplasm and be seized upon as another form of idioplasm, and that they should encounter in ergastoplasm and chromidia rival structures.

It is not, however, to offer any criticism of the mitochondria work on either its practical or theoretical side that reference to them is introduced here—the former would be gratuitous, the latter premature—but to offer from the standpoint of cytoplasmic fixation the comment that such morphological work takes too little cognizance of what kind of substances such a technique would be likely to preserve and bring out. The composition of the cytoplasmic fixers employed indicates that their efficiency depends upon the presence of reducing substances. In the usual technique, the bichromate with hydration is reduced furnishing to the reducing protoplasm a chromium oxid (?) which is there held. Where the substance involved is abundant and strongly reducing, the 'chromation' is immediate and the brown color assumed marked, as in the adrenalin (chromaffin) reaction; in other cases proceeding more slowly, or with scarcely detectable

color. Upon the chromation however the subsequent differential staining depends, involving therefore not only the reduction of the bichromate but the retention of it throughout the various steps of the technique. In passing it may be suggested that bichromate and chromic acid, although apparently opposite in their action as fixers—the first of good penetration, slow in its action, without marked power to precipitate proteins in an insoluble form; the latter poor in penetration, a rapid and energetic precipitant of protein—in last analysis must have the same action. Bichromate at its place of action in the cytoplasm (through reduction), takes on the fixing power of the chromic acid, its power of penetration being correlated with the indirectness of its action. Chromic acid on the other hand through its immediate energetic and extensive action as coagulant and oxidizer has its region of good fixation very superficial and its penetration correspondingly poor; its very good qualities as a fixer become thus its chief defects.

Theoretically while it would seem that an acid reaction in the fixing fluid would facilitate the action on proteins the oxidation and chromation would not be favored. In the case of the mitochondria, however, in many instances at least the reaction (chromation) seems to depend upon the presence of substances soluble in or changed by acids, strong alcohols, chloroform, ether, etc. Such reducing substances are found among the lipoids. Regaud recognized the similarity of method for the demonstration of mitochondria and for lipoids and therefrom suggested the chemical nature of the former. Fauré-Fremiet, Mayer and Schaeffer ('10) later made an extensive study of the matter and have pointed out that the mitochondria quite evidently embody a lipoid constituent in their make-up, the evidence being briefly: (1) their destruction, partial or complete, by fat solvents, (2) their preservation by the same chemical substances (potassium dichromate, chromic acid, osmic acid, formaldehyde; compare the Weigert method, etc.), (3) similarity in their staining reactions, (4) their similar staining by means of 'intra-vitam' stains (cf. Michaelis, '00). Fauré-Fremiet and Prenant regard the mitochrondria as composed of Lecith-albumins. There seems, however, from the conditions of the method no reason why lipoid substances of quite different

character might not be demonstrable by the method, provided they can meet the conditions, namely, the reduction and retention of the chromate.[3]

Indeed it is quite conceivable that other reducing substances (e.g., in the case of the histogenesis of the neuro-fibrillae?) may satisfy the conditions of the method. It is only necessary to recall the so-called 'Chromaffin Reaction' in the 'adrenalin granules' which are demonstrable by the modified mitochondrial technique (as, for example, applied by Meves).

As against the morphological significance claimed for the mitochondria as a distinct class of cell organs, handed down from cell generation to cell generation, may be urged:

1. The variability in their form, hence the multiplicity of names coined for their designation (mitochondrium, chondriomitoma, chondriokont, plastochondrium, chondriosoma). This in itself would not be a point of much weight. No adequate study has as yet been made, so far as the writer knows, upon the variability in form in the same kind of cell under different modifications of the method (cf. Schaxel, '11). In the egg cells investigated by him under the Benda treatment rod forms predominated, by the Altmann method, granules). The question of what is to be regarded as the more primitive form is involved; Meves found rods in the embryonic cells in the chick, Rubaschkin granules in the embryo guinea pig, etc.

2. Their apparent inertness and the indifference with which they are treated when the cell divides.

3. The unsatisfactory evidence of their mode of formation. Do they come from preëxisting mitochondria, or are they formed de-novo, appearing in the midst of the cytoplasm independently of other 'mitochondrial substance'? Are they directly or indirectly derived from chromatin?

4. Finally, the principles and conditions of the technique itself. Is every granule, row of granules, rod of thread brought out by a 'mitochondrial method' to be regarded as mitochondrial; if not, where and how is the line to be drawn?

[3] Faure-Fremiet, Mayer and Schaeffer seem to consider the oxidation of the fatty acid to hydroxy-acids much less soluble than the original substances, as equally important in the technique.

On the other hand, from the view-point of the nature of the substances demonstrable by the method, the results of the studies carried out by the application of this group of methods is very suggestive. A few points may be mentioned: (a) the relation to the nucleus and the intricate problem of nuclear synthesis; (b) possible significance of the clustering of 'mitochondrial granules' and chondriosomes around the centrosomes and idiosome in the spermatocyte and oocyte, as about a 'reduction center' in the cell;[4] their appearance or increase during the growth of the spermatocyte; (c) the introduction of reducing substances (lipoids?) into the egg in the spermatozoon in fertilization in the light of the experimental work of Loeb and others; (d) finally, the association of cytoplasmic activity, especially on its formative side with reduction processes. This might seem the broadest aspect of the results of the application of oxidizers in the fixation of cytoplasm.

That living substance is strongly reducing is of course well known, and the undoubted association therewith of the need of oxygen in respiration, the reducing power being in some instances great enough to permit protoplasm to obtain the oxygen by breaking up relatively stable compounds. Equally well known is the fact that with increased activity in protoplasm, at least on the formative and movement sides, there is an increased demand for oxygen. Save, however that the need of oxygen is inherent in life activity, and that the nucleus is in some way directly or indirectly concerned in its utilization, little definite seems to be settled. Reference may be made to the Hoppe-Seyler theory and its modification offered by Mathews ('05), briefly, that in protoplasmic activity reducing substances are formed for whose neutralization oxygen is necessary.

It is therefore suggestive that by the application of a technique calculated to bring to demonstration substances possessing reduc-

[4] Compare Lillie's theory of cell division. In such a distribution of electrical potentials as the theory postulates, reducing substances (characterized by H+) would accumulate around or tend to move toward the negative center (idiosome and centrosomes) there to deposit their positive charges; oxidative substances (characterized by OH−) would tend to concentrate around the nucleus (chromosomes).

ing power, the mitochondrial work in a steadily increasing list of contributions indicate the close association of the mitochondria with formative forces in the cytoplasm, yolk formation, secretory activity, histogenesis of muscle, nerve, connective tissue, embryonic growth, etc.

Since the suggestive paper of Ehrlich in 1885 in which he called attention to the need for oxygen and the reducing power of the cells and tissues, little study has been devoted to the basis of intracellular or tissue respiration on the histological side. Unna ('11) has recently made an analytical study of the localization of reduction and oxidation in the body, and has shown by the application of such oxidizers as potassium permanganate, ferric chlorid and potassium ferricyanide, and tetranitrochrysophanic acid, that the cytoplasm is strongly reducing while the nucleus is apparently not at all so but oxidative instead.[5] This is perhaps what was to be anticipated and accords well with the results of cytoplasmic fixation. Cytoplasm as a whole appears to possess reducing power, hence the usefulness of the fixers employed by Bensley ('10) in preserving the general morphology of the cell body. Unna's table (p. 14) is very suggestive of the association of strong reducing power and protoplasmic activity and throws added light on cytoplasmic fixation and the mitochondria problem. If a close connection exists between reduction processes and cytoplasmic (protoplasmic) activity and if there is a parallel between protoplasmic activity and the demand for and consumption of oxygen in respiration, and the mitochondria are the structural expression thereof, the question will at once be raised as to the nature of the reducing substances, and it will be pointed out that the reducing substances that are present in cell respiration may far exceed in power the lipoid combinations that are believed to be present in the mitochondria. To this two comments may be offered: (a) That whereas lipoid substances seem to be back of the mitochondrial reaction in some cases, it by no means follows that the reaction is due in every case to such substances, nor does it follow that structures demonstrated as mitochondria are in all instances

[5] Cf. Kingsbury, Histological Technique, 1910, p. 3.

closely connected with cytoplasmic respiration. (b) That since in regions of the body where the reducing power is markedly developed such as the medulla of the suprarenal gland, the myelinic nerve fiber and the red blood corpuscle, there is an association with lipoids, it may well be that such substances are more intimately and universally connected with the reduction processes of the body than would at first appear.

In conclusion—it is felt, therefore, that while the application of cytoplasmic fixers affords a preservation very useful in the study of the processes that take place in the cell body; on the morphological side, great hesitancy should be shown in drawing conclusions from such special technique, the first question being as to the nature of the substances upon which the method depends; the second, the part the substances or structures play in the cell economy. A case in point which illustrates the danger, as the writer believes, of basing morphological generalizations upon special technique without first ascertaining upon what the technique depends is found in the so-called 'chromaffin reaction' (cf. Kingsbury, '10). In the case of the mitochondria, if they are considered as constituting a distinct group of structural elements in the cytoplasm, the same criticism holds. In the interpretation of such structures in the cytoplasm, the issue is thus between a process interpretation of structure as against an 'elementary particle' or material, interpretation. The latter is, of course, the dominant one today as in the past. Cells, chromosomes, biophores, biogens, bioblasts, the emphasis is on structural units which are grouped together to form structure. Almost of necessity the elementary particle interpretation embodies the idea of superior grades of protoplasm, together with that of dominance or control, introducing thus an element of mysticism.

The process interpretation sees in structure the material expression of a 'play of forces,' present or past. The emphasis is here on unity; the key-note being change,—process. In the cytoplasm where diversity of structure and activity are more easily correlated, a process interpretation finds readier application. A transfer of such interpretation to the nucleus were in these days of personification, not to say idolatry of chromatin, bold indeed;

still, it might be ventured that as long as, for example, 'conjugation' of chromosomes is dealt with as though they were entities with independent power of movement, instead of the processes back of it, the superstructure of theory must remain as unwieldy as at present.

Aside from the question of the interpretation of appearances produced by the application of this class of fixing fluids, or their use when it is desired to bring out certain definite substances or structures (rational fixation), there remains the purely empirical fact that when it is desired to preserve well the cytoplasm, particularly in more difficult cells such as gland cells, the use of these fixers is indicated. Of the formulae devised, Helly's fluid, or Zenker-formol has the general preference by the writer (cf. however, Bensley '11), 10 per cent instead of 5 per cent of formalin being added to the Zenker stock solution. Recently copper dichromate has been substituted for the potassium dichromate with good results, the bichromate salt of the heavier metal, being, as Bruckhardt showed, superior to the potassium salt as a chromatin fixer. As furthermore the Weigert copper hematoxylin stain is a favorite one, the copper fixation is especially suitable in preparation. A formula giving good results is: 10 per cent copper bichromate, one part; 4 per cent copper sulphate, one part; saturated aqueous solution of mercuric chlorid, two parts; formalin, 10 per cent. Since, like Helly's fluid, the mixture is unstable, it must be made up from stock solutions as needed. Fixation should not proceed for more than twenty-four hours, when, after rinsing, the tissue may be transferred to a simple copper bichromate solution ($2\frac{1}{2}$ per cent), if further copper mordantage is desired. Three days will give sufficient mordantage for an excellent Weigert stain of the nervous system. Where the best preservation of cytoplasmic form is desired, Bensley, as has been stated, recommends that the formalin be first freed from acid by careful neutralization and redistillation. In general, a small amount of acid, giving an acid reaction, seems to be favorable to the fixation, particularly where the preservation of fats is in question. This may be secured by adding two or more drops of glacial acetic acid per 100 cc.

Heidenhain's iron hematoxylin is an excellent stain, though copper hematoxylin has some advantages, while Meves' recent study on Ascaris shows how excellent is the original Altmann technique as a mitochondrial stain.

The above fixers are not, of course, universal cytoplasmic fixers. In fact, astral rays and spindles in cell division seem particularly poorly differentiated. This is possibly of itself an indication that the definite fibers are a residuum and owe their clearness to this fact.

As to the chromidia vs. mitochondria controversy the comment may be offered that there should be no difficulty in differentiating nucleo-protein granules from those specially brought out by the employment of the fixers discussed in this paper, the application of Unna's granoplasma technique, among others, should preserve the former and not the latter. There is however no a priori reason known to the writer why cytoplasmic granules should not be of composite character and embody a nucleo-protein as well as a lipoid constituent, as indeed seems sometimes to be the case, and be differentially stained by both methods.

It is however in the fixation and not in the staining that such differentiation should be sought. It is only on the basis of an 'elementary particle' interpretation that such a distinction of chromidia from mitochondria would have particular value, a process interpretation would not emphasize distinctions, but relations.

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ON THE WEIGHT OF THE CRANIA OF NORWAY AND ALBINO RATS FROM THREE STATIONS IN WESTERN EUROPE AND ONE STATION IN THE UNITED STATES

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In the course of some observations on the relative weight of the central nervous system in Norway and albino rats from three stations in western Europe and one station in the United States (Donaldson, '12) the dried crania from nine series were weighed. All of the measurements and records applying to these series are to be found in the paper just cited and are therefore not repeated here.

The data thus obtained are of interest from several points of view:

First: As illustrating the difference in the weight of the crania between the wild Norways and the domesticated Albinos.

Second: As bearing on the relation between weight of cranium and cranial capacity.

Third: As showing the relation of weight and density of the cranium to shrinkage on drying.

Fourth: As revealing the increase in the weight and capacity of the cranium in Albinos of the third generation reared at a high temperature.

In order to determine the weight of the cranium[1] there were taken when possible from each series six crania (three from males

[1] Cranium = skull with teeth—minus mandible.

and three from females)[2] for each body weight group beginning with a body weight of 125 grams and rising by 50 gram increments to 375 grams, thus giving six groups—50 grams apart.

The foregoing arrangement was made for the four series of Norway rats (Mus norvegicus, all freshly caught) coming respectively from Vienna, Paris, London and Philadelphia.

When the average weights of the six skulls representing the several body weight groups are tabulated for the four series, we obtain the values given in table 1.

TABLE 1

Giving the mean weight in grams of the crania in each body weight group for the four series of wild Norway rats from London, Paris, Philadelphia and Vienna. Those instances in which the female crania are heavier than the male are underlined

BODY WEIGHT GROUP	WEIGHT OF THE CRANIA IN GRAMS			
	London	Paris	Philadelphia	Vienna
grams				
125	1.17	1.27	1.13	1.10
175	1.58	1.58	1.34	1.37
225	1.84	1.91	1.71	1.70
275	2.25	2.17	2.14	1.90
325	2.69	2.60	2.40	2.27
375	3.13	2.98	2.86	2.48
Average for all body weight groups: 250 grams	2.11	2.09	1.93	1.80
Deviation from Philadelphia average at 250 grams taken as a standard	+9.3 per cent	+8.3 per cent	Standard	−6.7 per cent
Average for the first three body weight groups: 175 grams	1.53	1.59	1.39	1.39

[2] The value used for the final average of the body weight group was the average of the weights for each sex group, even when the number of the individuals in the sex group was *less than three*. The determinations thus made were plotted on a chart for the body weight to which they applied and then the values corresponding to the body weights 125, 175, 225, 275, 325 and 375 chosen for the tables were read from the graph. As the graph is practically a straight line for both cranial weight and cranial capacity, this method can be used without introducing any significant error.

It should be noted in passing that slight fluctuations of as much as 0.5 per cent may occur in the weight of thoroughly dried crania due to the variations of temperature and of moisture in the surrounding air to which the organic constituents of the crania respond.

Table 1 shows (see averages) that the several series of crania have not the same mean weight; the London crania being heaviest and those from Vienna lightest. The divergence of the records for the different series tends to be more marked in the heavier body weight groups. The average weight of the crania in the three European series combined is however greater than in the Philadelphia series.

In the cases where the female skulls were heavier than the male, the entry is underlined. As will be seen, this occurs in 16 out of the 24 entries, and more frequently for the smaller body weights. Like so many of these sex relations, this one appears to depend mainly on the fact that for a given body weight the female is older and therefore has a relatively heavier cranium—so long as the female cranium is actively growing—but since growth becomes slow in the female before it does in the male, the male cranium is usually the heavier in the heaviest body weight groups. To facilitate comparison with the Albino records (tables 7 and 8), which are to be considered later, the average values for the first three entries in each series are also given in table 1.

If we turn now to the capacity of these crania in cubic centimeters, we obtain from the collection of crania used for table 1, the data given in table 2.

This shows that the European crania are much alike in capacity but the several averages of the European series agree in being from 1.4 per cent to 3.9 per cent below the Philadelphia series. There is moreover a lack of complete correspondence between weight of cranium and cranial capacity as is shown in table 3, in which two series of data are compared. As in the case of table 1, and for the same reason, the averages for the first three entries are also given in table 2.

The direct weighings of the fresh brain in the case of the Paris and of the London Norway rats show the former to be 2.0 to 2.9

TABLE 2

Giving the mean capacity in cubic centimeters of the crania in each body weight group for the four series of wild Norway rats from London, Paris, Philadelphia and Vienna. The instances in which the female crania have the greater capacity are underlined

BODY WEIGHT GROUP	CAPACITY OF CRANIUM IN CUBIC CENTIMETERS			
	London	Paris	Philadelphia	Vienna
grams				
125	1.75	1.75	1.84	1.70
175	1.90	1.89	1.87	1.82
225	1.98	2.00	1.99	1.93
275	2.05	2.10	2.10	2.02
325	2.09	2.21	2.25	2.14
375	2.23	2.24	2.33	2.25
Average for all body weight groups: 250 grams	2.00	2.03	2.06	1.98
Deviation from the Philadelphia average for 250 grams as standard	−2.9 per cent	−1.4 per cent	Standard	−3.9 per cent
Average for the first three body weight groups: 175 grams	1.88	1.88	1.90	1.82

per cent above, and the latter almost the same as, those from Philadelphia (Donaldson, '12). There were no observations on the fresh brains of the Vienna rats.

It follows from this that the smaller cranial capacity of the several European series as indicated in table 2, must be due to the fact that the European crania have shrunk more in drying than have the Philadelphia crania. The possibility of such differences in shrinkage lies in the considerable range in composition and thickness of the skull bones, to be inferred from the general studies of Wildt ('72), Weiske ('89) and Watson ('06) on the composition of the bones.

Such shrinkage is due to loss of water. We might expect light, thin crania like those of the Vienna series to shrink more than the heavier crania, but we have the striking instance where the Phila-

TABLE 3

Comparison of cranial weight and cranial capacity in the four Norway series. In each group the entries are arranged in the order of diminishing values. Both series of means apply to a rat with a body weight of 250 grams.

STATION	CRANIAL WEIGHT	CRANIAL CAPACITY	STATION
	grams	*cc.*	
London	2.11	2.06	Philadelphia
Paris	2.09	2.03	Paris
Philadelphia	1.93	2.00	London
Vienna	1.80	1.98	Vienna

delphia series, composed of rather light crania, has shown the least shrinkage, while the London crania, which are the heaviest, have shrunk to about the capacity of the Vienna series—yet by direct observations (see above) it is shown that the Paris and London rats yield brains as heavy, or a trifle heavier than those of the Philadelphia series.

The point here made therefore is that the amount of shrinkage is not controlled solely by the weight of the skull—but that the factor of density must also enter in. In favor of this view, some slight evidence has been already obtained, but the matter requires more extended study before it can be fully discussed.

In the case of the albino rat (Mus norvegicus albinus) it is desirable to make so far as possible, comparisons similar to those we have made in the case of the wild Norway. Unfortunately however, the number of specimens from the several European stations is small, and hence the determinations are less precise, though still worth consideration. Table 4 gives the mean weight in grams of the crania in the several series.

Table 4 shows, in the averages of the first three entries of each series, mean values which decrease from the Vienna records to the London records—following exactly the reverse order to that of the corresponding records for the Norways (table 1).

It appears therefore that similarity of station is not associated with a similar relative development in cranial weight in these two forms.

TABLE 4

The mean weight in grams of the crania in each body weight group of the four series of albino rats from Paris, London, Philadelphia and Vienna

BODY WEIGHT GROUP	WEIGHT OF CRANIA IN GRAMS			
	London	Paris	Philadelphia	Vienna
grams				
125	0.89	1.03	1.05	1.00
175	1.23	1.27	1.41	1.40
225	1.52	1.52	1.51	1.73
275	1.79		1.87	2.10
325			2.15	
Average of the first three entries in each case: 175 grams	1.21	1.27	1.32	1.38

A comparison of the absolute cranial weights can be made only by taking from table 1 an average of the first three entries of all four series combined—to be compared with the corresponding data from table 4. This gives the following:

Average of the averages for the first three entries in all series in table 1 (wild Norways)..... 1.475 grams
Average of the averages for the first three entries in all series in table 4 (domesticated Albinos)..... 1.295 grams
Difference = 0.180 = 13.9 per cent in favor of the wild Norways using the Albino value as the standard.

It appears from this that in the case of the Norway rat weighing 175 grams, which we may consider the half grown, the average weight of the crania is some 14 per cent greater than in the Albinos of like body weight.

This value would probably be increased if the comparison could be made with the higher body weight groups.

When these several series of Albino crania are compared as regards capacity, it is seen (from table 5) that the differences are comparatively slight and that the order of decreasing capacities runs quite differently from the order of decreasing cranial weights.

TABLE 5

The mean capacity in cubic centimeters of the crania in each body weight group of the four series of albino rats from Paris, London, Philadelphia and Vienna

BODY WEIGHT GROUP	CRANIAL CAPACITY IN CUBIC CENTIMETERS			
	London	Paris	Philadelphia	Vienna
grams				
125	1.48	1.59	1.49	1.43
175	1.63	1.70	1.67	1.62
225	1.74	1.85	1.76	1.73
275	1.78		1.81	1.87
325			1.87	
Average of first three entries in each series: 175 grams	1.62	1.71	1.64	1.59

TABLE 6

Comparison of cranial weight and cranial capacity in the four Albino series. In each group the entries are arranged in the order of diminishing values. Both series of means are based on the first three entries only in tables 4 and 5, and thus apply to a rat with a body weight of 175 grams

STATION	CRANIAL WEIGHT	CRANIAL CAPACITY	STATION
	grams	*cc.*	
Vienna	1.38	1.71	Paris
Philadelphia	1.32	1.64	Philadelphia
Paris	1.27	1.62	London
London	1.21	1.59	Vienna

The lack of correspondence between the two series of data as shown in table 6 is similar to that found in the case of the Norway rat (see table 3) only more marked. Thus in the several series for both forms changes in cranial capacity do not regularly correspond with changes in cranial weight.

This lack is still more strikingly shown when we compare the average values of the first three body weight groups of the Albinos from Philadelphia and Vienna, table 4, with the values for the same groups of Norways from the same stations (see table 1). The values used are given in table 7.

TABLE 7

Comparing for the first three body weight groups of both the Norway and Albino series from Philadelphia and Vienna, the averages for the cranial weight and cranial capacity

	MEAN BODY WEIGHT	MEAN CRANIAL WEIGHT	MEAN CRANIAL CAPACITY
	grams	*grams*	*cc.*
Norways	175	Philadelphia from table 1...1.39	1.90 from table 2
		Vienna from table 1.....1.39	1.82 from table 2
Albinos	175	Philadelphia from table 4..1.32	1.64 from table 5
		Vienna from table 4.....1.38	1.59 from table 5

It is evident from table 7 that the cranial weights are only slightly less in the case of the Albinos, while the capacities are much less. In these cases the measurements of the crania in length, breadth and height after the method of Hatai, '07, give results in accord with the cranial capacities. It follows from this that in these series as given in table 7, the Norways have thinner crania than the Albinos.

When however a similar comparison is made between the Paris and London Norways and Albinos, the relations are reversed in the sense that the cranial weights of the two forms differ much more than the cranial capacities. When finally the comparison is made for the entire four series of each form, we obtain the following result:

Average of the averages for the first three entries in all series in table 2 (wild Norways)..... 1.87 cc.
Average of the averages for the first three entries in all series in table 5 (domesticated Albinos)..... 1.64 cc.
Difference = 0.23 cc. = 14.0 per cent in favor of the wild Norways using the Albino value as a standard.

Thus the percentage difference in capacity in favor of the Norway is almost identical (see p. 58) with the percentage difference in the cranial weight (13.9 per cent) when *all* the Norways are contrasted with *all* the Albinos.

While I was collecting rats in Vienna in 1909, Dr. Przibram kindly put in my hands a series of ten albino rats representing the third generation reared in the Vivarium at 95 to 100° F.

The crania from this series were prepared. They are unusually heavy. Table 8 will enable us to contrast the crania of these Albinos reared in the heat with the corresponding crania from both Vienna Norways and normal Vienna Albinos.

Two things are shown by table 8, first: the Albinos reared in the heat have by far the heaviest skulls; second: their cranial capacities are distinctly (5 per cent) greater than those of the normal Albinos, yet not so great as those of the Norways. This is rather a striking result and suggests that high temperature may be able to produce some marked changes. It is however possible that the greater cranial capacity in the 'heat' series does not mean an increase in the weight of the brain, but merely a lesser shrinkage in the heavier skulls. The present instance merely serves to indicate the influence of heat in a general way, as well as to show

TABLE 8

Comparing in respect to cranial weight and cranial capacity the Vienna series for the Norway, normal Albinos, and Albinos reared at a high temperature

BODY WEIGHT GROUP	CRANIAL WEIGHT IN GRAMS		
	Norways (from table 1)	Normal Albinos (from table 4)	Albinos reared in heat
125	1.10	1.00	1.25
175	1.37	1.40	1.67
225	1.70	1.73	2.14
Average for all groups: 175 grams	1.39	1.38	1.69 =+22 per cent

BODY WEIGHT GROUP	CRANIAL CAPACITY IN CUBIC CENTIMETERS		
	(from table 2)	(from table 5)	
1.25	1.70	1.43	1.52
1.75	1.82	1.62	1.66
2.25	1.93	1.73	1.84
Average for all groups: 175 grams	1.82	1.59	1.67 =+5 per cent

once more that while increase in cranial weight (22 per cent) is here accompanied by increase in cranial capacity (5 per cent) the former change is much more marked than the latter, and indeed the latter cannot at the moment be safely interpreted.

I have measured the length, breadth and height of the crania in these two series and found that the volumes represented by the products of these three diameters are so related that the mean value of those for the 'heat' series is just 4.9 per cent above that for those of the control series, thus agreeing as it should, almost precisely with the direct observations on the difference in capacity.

It can be further stated that when these two series of crania were dried at 95° C. for four days (ninety-six hours) the normal crania lost 7.86 per cent in weight and the crania of the heat series 7.70 per cent or very nearly the same amount. This indicates that the composition of the bone in the two series is similar in the relation of salts to organic matter. The heavier (heat) crania are therefore heavier because they contain more bone of the same general composition as that in the crania of the normal series. We conclude therefore:

1. That while the Norways have absolutely heavier crania than the Albinos, as would be expected, yet when the entire groups of Norway crania is compared with the entire group of Albino crania, the difference in cranial weight is in proportion to the difference in cranial capacity. Therefore the bones composing the crania are similar in mean thickness in the two groups.

2. When the two forms from each station are compared series by series, the Norway crania are always somewhat heavier than the Albino crania, but the correlation with cranial capacity is low.

3. The differences in the amount of shrinkage of the crania on drying are referred to differences in the thickness of the bones and the amount of organic matter in them.

4. The Albinos reared at 95° F. by Dr. Przibram show as compared with the normal Vienna Albinos a remarkable increase of 22 per cent in the weight of the 'heat' crania—a striking modification—and also some increase in cranial capacity (5 per cent) but the interpretation of this latter result cannot at the moment be made.

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A MICROCHEMICAL STUDY OF THE FATTY BODIES IN THE INTERSTITIAL CELLS OF THE TESTIS

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In a paper published several years ago ('08) I stated, apropos of Ganfini's theory that the fatty contents of the interstitial cells of the testis constitute an internal secretion which reaches the blood through the lymphatics, that no sufficient evidence had been offered to show that this fat differs from ordinary neutral fat. Microchemical methods had not been sufficiently developed at that time to allow differential detection of the various fatty substances. Since then, however, the activity in this line of investigation has been great, and the histologist may now hope to orient himself, roughly at least, among the various classes of fatty substances which the chemist has described. Accordingly it has seemed worth while to study the fatty contents of the interstitial cells by the new methods.

Marked progress may be said to have been begun by the studies of Smith, Mair and Thorpe ('08, '11) in the course of which they worked out the rationale of the staining of medullary sheaths of nerves by Weigert's haematoxylin method. They found, in brief, that during the mordanting by the potassium bichromate employed as a fixative the unsaturated fatty acid constituents of the fatty substances in the sheath are oxidized. A molecule of chromium oxide unites with each molecule of unsaturated fatty acid with a twofold result—the fatty substances are rendered insoluble in the usual dehydrating and clearing fluids of the laboratory, and the chromium-fatty acid compound gives a lake with haematoxylin when the sections are subsequently stained with that dye. They found, furthermore, that there is a certain optimum time for the reaction to occur: if the mordant-

ing was not carried far enough, or if it were carried too far, the chrome-haematoxylin lake was not formed—it is only during the process of oxidation that the union between the chromium oxide and the unsaturated fat occurs in such a way as to produce a lake with haematoxylin. They were thus led to experiment with various fatty substances, either in the tissues, or in the pure state soaked up by bits of cigarette paper. It was found that there were great differences in the readiness with which the reaction could be obtained with different fatty substances. For example, neutral fat in frozen sections of fatty liver or pericardium did not give the reaction until the sections had been exposed to the action of a saturated solution of potassium bichromate for two weeks at the temperature of 37° C.; while, on the other hand, mixtures of cholesterin and fatty acid, and of cholesterin and lecithin (Kahlbaum) were oxidized very readily.

Ciaccio ('09) as the result of his study of the action of potassium bichromate upon various fatty substances devised the following method: Small pieces of tissue are fixed twenty-four to forty-eight hours in a liquid composed of 5 per cent aqueous solution of potassium bichromate 100 cc., formalin 20 cc., and acetic acid 5 cc. (or formic acid ten to fifteen drops). After fixation the tissue is mordanted in 3 per cent solution of potassium bichromate for about a week at 37° C., or a little longer at room temperature. After washing twenty-four hours it is imbedded in paraffin in the usual way, except that the time devoted to dehydration and clearing is lessened. The sections are stained first with Sudan III, and then by iron alum haematoxylin. The Sudan is relied upon to stain any fat present, while the haematoxylin gives a good counter stain, and also demonstrates certain cellular structures, e.g., mitochondria. Ciaccio held that neutral fat, fatty acid, and cholesterin after this treatment would be dissolved out of the tissues during the processes of dehydrating and clearing, and that the globules demonstrated by Sudan III were lecithin; and he proposed therefore to call cells containing such substances lecithin cells.

Ciaccio's method is doubtless open to criticism in some particulars. Fauré-Fremiet and his collaborators ('10) have pointed

out that unsaturated fatty acids are demonstrated by it, and Kawamura ('11) adds that soap (sodium oleate) and a mixture of cholesterin with the phosphatid lipoid cephalin are also stained by it. Moreover, it is commonly believed that pure lecithin does not exist in nature; indeed, chemists seem quite undecided as to just what mixture of substances constitutes lecithin (Brigl, '11). Ciaccio himself in a later publication ('10) suggests that it might be more appropriate to speak of lipoid cells rather than lecithin cells. Bell ('11) found that triolein could be stained to some extent after treatment by the method, omitting imbedding in paraffin. Bits of cigarette paper when saturated with triolein and thus treated showed some small homogeneous droplets stained by the Sudan. Bell's study indicates that the smaller the droplet the more readily it is chromated; so that small droplets of this neutral fat in tissues might be demonstrated by Ciaccio's method. In spite of these objections my experience with the method leads me to think that, taken in conjunction with other tests, it will be found very useful in eliminating certain fatty bodies from consideration. To Smith and Mair ('07, '10, '11) we are indebted for yet another useful method. These observers have found that the basic dye Nile blue sulphate, which stains fatty acid blue, stains neutral fat red. On chemical investigation they determined that this reaction was due to the spontaneous development in watery solutions of Nile blue sulphate of a new dye, red in color and soluble in liquid fat. So that a watery solution of this dye contains really a double dye—a blue dye basic in character, and a red dye, developed from the base but non-basic in character, which imparts a red color to neutral fat. Nile blue sulphate thus offers a means of differentiating between neutral fat and fatty acid, in the sense that a given globule is stained blue if it consists of fatty acid, red if of neutral fat, and an intermediate color if it be a mixture of the two. Smith and Mair recommend a saturated aqueous solution to which one-half per cent sulphuric acid is added in order to hasten the formation of the red dye. Frozen sections are stained over night and differentiated in two per cent acetic acid.

Fauré-Fremiet, Mayer and Shaeffer ('10) in the course of a study of the chemical nature of mitochondrial formations sub-

jected various fatty substances in a pure state to numerous procedures. Their results are incorporated in tables which will doubtless be valuable to others pursuing similar investigations.

The most extensive investigations of this sort, however, have been made by Kawamura ('11). He has experimented with a large number of pure substances, both singly and in mixtures, with results which seem likely to be distinctly helpful. The substances examined were soaked up by cigarette paper, which could then be handled like frozen sections—the so-called Wlassak method. He has made particular use of the methods of Smith and Mair as well as that of Ciaccio applying them, as stated above, to a large number of fatty bodies. It is not necessary to enter into the details of this monograph here, but I shall have occasion to refer to some of his results later on. Among other things, however, he found that Nile blue sulphate stained many fatty substances either red or blue which are neither neutral fats nor fatty acids; he also made extensive studies of the optical properties of fats.

It thus appears that there are sufficient data at hand to justify an attempt to identify the fatty globules contained in the interstitial cells of the testis. So far as I am aware, no such study has been made except by Ciaccio ('10). He included the interstitial cells among the various fat-containing tissues and organs which he investigated by his methods, and concluded that they should be classed with his 'lecithin cells.'

Before beginning my own study it seemed advisable to investigate the insolubilizing effect of potassium bichromate on neutral fat, in view of the conflicting statements on that subject in the literature. Smith and Mair found that frozen sections of fatty liver and pericardium would not give the chrom-haematoxylin lake until they had been in a saturated solution at 37° C. for two weeks. They do not state specifically that the fat in sections thus treated was rendered insoluble, but in view of their explanation of the action of potassium bichromate on fatty bodies the inference that such was the case seems justifiable. Fauré-Fremiet and his collaborators say that neutral fat is unaffected by treatment with potassium bichromate. Ciaccio and Kawamura state that after treatment by Ciaccio's method neutral fat is dissolved

out in the processes of imbedding. Bell, on the other hand, could demonstrate globules of triolein in cigarette paper treated by Ciaccio's method, omitting only the actual imbedding in paraffin.

I have attempted to test this point under conditions comparable to those affecting fat in *blocks of tissue* in the following way: Some triolein emulsified in a weak solution of egg white was injected with a hypodermic syringe into the thigh muscles of a freshly killed frog, and immediately afterwards the preparation was immersed in a 10 per cent solution of formalin. It was assumed that in this way any decomposition of the fat in the muscle would be prevented. Frozen sections stained with Sudan III showed that the oil was fairly well distributed through the muscle bundles. For the most part it was in masses of considerable size; but numerous globules, some of them quite small, were also present. Blocks of the muscle 3 or 4 mm. thick were treated by Ciaccio's method, and imbedded in paraffin in the usual way, i.e., not shortening the time ordinarily devoted to dehydrating and clearing (the blocks were left in absolute alcohol twenty-four hours, and in xylol twelve hours). In sections of the muscle thus prepared absolutely no fat was demonstrated by staining a half hour in Sudan at 37° C. When blocks were left in 3 per cent bichromate for three weeks at a temperature of 37° C., considerable quantities of fat stained with Sudan. Practically the same results were obtained with human subcutaneous fat placed while still warm in 10 per cent formalin solution. In frozen sections the globular masses of fat in the adipose cells stained rose-red with Nile blue sulphate. In sections of the tissue prepared by Ciaccio's method no fat could be demonstrated by Sudan. When the tissue was left in bichromate for two weeks, however, a few small grains were stained by Sudan at the periphery of the block. I conclude therefore, with Smith and Mair, that unsaturated neutral fat (triolein) is acted upon by the bichromate very slowly, but that the final result is the same, it being largely a matter of time, other things being equal. I also conclude, with Ciaccio, that it is improbable that the globules of fatty substances found in the interstitial cells of the testis after fixation and treatment by Ciaccio's method are composed of neutral fat, as such fat is not sufficiently

insolubilized by the length of chromation to prevent it from being dissolved out in the fluids employed in dehydrating and clearing.

In this study I have found Ciaccio's method very useful, largely because it enables us to eliminate a number of fatty substances. As we have seen, the globules demonstrated by this method are not neutral fat, as such fat would in all probability be dissolved out of the tissue during the processes of imbedding. According to Kawamura, we can also eliminate for the same reason the esters formed by fatty acids with cholesterin, mixtures of fatty acid and cholesterin, and the non-phosphatid lipoids (cerebroside). So that, theoretically, the globules demonstrated by Ciaccio's method may be one or more of the phosphatid lipoids, fatty acid, or soap, singly or mixed together. Ciaccio thought that his method also eliminated fatty acid and soap, but according to Fauré-Fremiet and Kawamura, he was mistaken in this. To determine the presence or absence of fatty acid and soap I have made use of the test devised by Fischler ('04), which consists, briefly, in converting any fatty acid or soluble soap which may be present into the insoluble lime soap, which, on treatment with acetate of copper, yields a lake with haematoxylin when stained by Weigert's method for medullary sheaths. Employed on frozen sections this test gave me negative results.

Thus by the process of elimination we reach the conclusion that the bulk of the fat contained in the interstitial cells of the cat's testis consists of a member, or perhaps of several members, of the group of phosphatid lipoids.

Is lecithin, which is frequently classed as a phosphatid lipoid, present? Ciaccio, as we have seen, thought that the bulk of the fatty bodies demonstrated by his method were composed of lecithin. Our knowledge of lecithin in the tissues is so extremely unsatisfactory that it hardly seems possible to give an answer to the question; still I have used the tests recommended by Smith and Mair ('11) for this substance. These authors state that pure egg-lecithin (Kahlbaum) is stained by acid fuchsin even after chromation. In my experiments with frozen sections of the cat's testis the globules were not stained by treatment with a one per cent aqueous solution of acid fuchsin even after two hours in the

incubator at 37° C. Sections of Ciaccio material stained in Altmann's solution of acid fuchsin for forty-eight hours in the incubator and differentiated by Altmann's method showed the mitochondrial formations stained a deep red, but the fatty globules were either unstained or colored light yellow—a color which may have been due to the picric acid employed in the differentiating fluid. Smith and Mair also state that lecithin stains with difficulty by Weigert's haematoxylin method, but stains very readily if a slight admixture of cholesterin is present. I find that the globules in the interstitial cells give the chrom-haematoxylin lake after two days' chromation, although, as will be seen later, it is probable that free cholesterin is not present in the globules. So far as these experiments go, pure lecithin seems not to be present in the globules; but for the reason previously stated I am not inclined to attach importance to these results.

Assuming, then, that the bulk of the fatty globules in the interstitial cells is furnished by a member or, perhaps several members, of the phosphatid lipoid group, is there any evidence to indicate that other fatty substances are present in accordance with the common observation that fatty bodies in the tissues are usually mixtures? An examination of frozen sections as compared with sections of Ciaccio material revealed some differences between the two. In the first place, the number of globules per cell seemed to be greater in frozen sections. In sections of the Ciaccio material cells with vacuoles or ring-shaped bodies were not very rare, indicating that some of the fat had been lost. Again, when frozen sections were examined with the micro-polariscope cells containing anisotropic globules either singly or in small groups were found with considerable frequency, whereas in the Ciaccio material no anisotropic bodies could be seen. In the latter material either the anisotropic globules had been dissolved out, or their optical properties had been changed. According to Kawamura's extensive study of the optical properties of fatty substances, the anisotropism here may have been due to the presence of cholesterinester or to a mixture of cholesterin with a fatty substance, especially fatty acid. To determine whether the cholesterin was present as an ester or uncombined I have had recourse to the

microchemical test for free cholesterin put forward by Golodetz ('08), which is based on the observation that free cholesterin is stained blackish brown by a mixture consisting of five parts of concentrated sulphuric acid and three parts of 30 per cent formaldehyde. Frozen sections of the cat's testis treated on the slide with a few drops of this mixture gave negative results. Accordingly it seems probable that the interstitial cells contain cholesterinester in quite appreciable amount.

Finally, frozen sections stained with Nile blue sulphate present color reactions which indicate that the fatty globules are not all of the same composition nor all homogeneous: While most of them stain dark blue, some have a reddish tinge, and others have a blue periphery and a reddish center. Now Kawamura's studies show that this dye stains blue not only fatty acids but also some phosphatid lipoids; so that, having eliminated free fatty acid by Fischler's test, we may conclude that the blue globules of the frozen sections are identical with those demonstrated by Ciaccio's method. The red tinge may be due to the presence of either neutral fat, or cholesterinester, or of both.

In conclusion: This study indicates that the fatty globules of the interstitial cells of the cat's testis consist for the most part of phosphatid lipoid material, but that cholesterinester and neutral fat also are probably present.

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THE ANATOMY OF THE HEART OF THE INDIAN ELEPHANT

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The specimen upon which the following description is based was obtained from a small female elephant that died in this city during a spell of hot weather. The lack of facilities for dissecting so huge an animal and the obligatory haste made it impossible to study any topographical relations and was the cause of the destruction of many parts that may prove of interest. After the heart was removed it was placed into formalin and it remained there for eight months. When it was taken therefrom it was found impossible to estimate the extent of the pericardium, and the number of veins that led to the atria. It had been cut off too short to determine these points, but in other respects the specimen is in excellent condition and justifies a detailed description.

The literature is very inadequate and contains many erroneous and conflicting statements. With the exception of the three most recent articles, Vulpian et Philipeaux ('56), Watson ('71), Miall and Greenwood ('78), we find that the bibliography has mainly an historical interest. Unfortunately not all the treatises were accessible, but no effort was spared to trace all previous descriptions to their source. The literature will be discussed in the course of this article.

The general shape of the heart is like a broad short cone somewhat flattened in antero-posterior diameter.[1] The diaphragmatic face has the shape of an equilateral triangle. The auricles (auricular appendages) are very small compared with the size of the heart. This may be due to a greater degree of contraction of the auricles.

[1] In describing the isolated heart I follow the rule adopted by most authors, namely, to imagine it with its base upwards and the interventricular septum in a sagittal plane.

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A thick epicardium covers the whole heart hiding the coronary vessels and the external divisions of right and left ventricles, except near the apex where the surface is deeply indented. Watson corroborates the statement of Aelian (1765), Stukeley (1722) and Mayer ('47)[2] that the apex is distinctly bifid, but according to our specimen it would be an exaggeration to agree with these authors. The apex is formed by the left ventricle alone. A marked oedema around the coronary sulcus obliterates it in places. It is probably this curious oedema which Vulpian and Philipeaux mistake for fat, an error which I would have made had it not been for the incisions made through it. Miall and Greenwood say that the heart they dissected was perfectly destitute of fat, and that the findings of Vulpian and Philipeaux is the only instance in which any considerable quantity of this substance has been met with in any part of the elephant. The following measurements, taken after fixation in formalin, may prove of interest.

The weight of entire heart, 10.5 kilogram.

The greatest width (at a level where the coronary sulcus crosses the septum on the posterior surface), 30 cm.

The greatest length, including aortic arch, 50 cm.

The length of ventricles from coronary sulcus to apex on the posterior surface, 25 cm.

The length of ventricles from coronary sulcus to apex on the anterior surface, 32 cm.

The antero-posterior diameter, 24 cm.

These measurements bear out the statement made by previous authors, Meckel (31), Owen ('68), that the heart is broader than it is long, for if we omit the aortic arch the length would be about 28 cm. as compared with 30 cm., the width.

In order not to destroy the heart as a museum specimen and to allow of further investigation, it was cut open in the manner

[2] After looking up the original article of Mayer I found that Watson had misquoted him. His description, with which mine agrees, is as follows: "An dem Apex Cordis sind beide Ventrikel aeusserlich durch eine tiefe Crena geschieden, so dass der Ventriculus sinister die Spitze des Herzens allein bildet. Ob es von dieser Theilung nahe der Spitze des Herzens herruehrt, dass man von Aelian bis auf Conrad Gessner dem Elephanten Zwei Herzen zuschrieb will ich dahin gestellt sein lassen."

usually pursued by pathologists. The left side was opened by joining an incision through the aorta and the anterior wall of the ventricle to one through a right pulmonary vein and the posterior wall of the ventricle at the apex. The right side was opened by a similar triangular incision through the pulmonary orifice and inferior vena cava. In the specimen at hand the cavity of the right ventricle and conus arteriosus seems about twice as large as that of the left ventricle. This is probably due to the greater degree of contraction of the walls of the latter, and must be taken into consideration when comparing the following measurements:

The average thickness of the wall of the left ventricle is 5 cm. while the thickness of the right ventricle near the apex is 1.5 cm. increasing toward the pulmonary orifice to 2.3 cm. The interventricular septum measures 7 cm. in thickness.

The endocardium is grayish in color and smooth. In places it measures over 0.5 mm. in thickness and can easily be stripped off in two layers. Beneath it, the musculature of the ventricles shows a marked cross-striations of its fibers. Upon histological examination, however, it was found that these cross-striations have nothing to do with the usual cross-striations of cardiac muscle, but are merely folds caused probably by the great degree of shrinkage of the endocardium in the fixing fluid. There does not seem to be any marked difference between the cardiac muscle fibrils of the elephant and those of other mammals in respect to their structure or their size. No Purkinje fibers are visible to the naked eye in either ventricle and no histological examination was made for them.[3] Their invisibility may be due to three causes, viz., the thickness of the endocardium which hides the musculature in general, a deeper position of the Purkinje fibers and the action of the fixatives which destroys the difference in gross appearance between Purkinje fibers and heart muscle. Small bloodvessels in the endocardium were conspicuous.

The inner surface of the heart offers no marked peculiarities with the exception of the presence of numerous foramina venarum minimarum (Thebesii) throughout both atria and ventricles.

[3] Since writing this article a description of the Purkinje fibers in the elephant's heart by Manuilow ('11) has appeared.

Ever since the time of Haller (1757), who denied their presence, there has been a discussion as to whether these foramina are the openings of actual veins or merely depressions of the endocardium. Practically all the modern text-books take the non-committal attitude that some are and others are not openings of real veins. The author has frequently attempted by injection through the coronary arteries in the pig's heart to obtain an injection of these venae minimae of the ventricles but without success. To attempt injection methods in a heart that has been thoroughly fixed as is the case with this heart of the elephant, would be perfectly futile. A positive result may indicate a rupture of a bloodvessel for it takes upwards of 300 mm. mercury pressure and a negative result may mean an occlusion by a blood-clot. However, owing to the fact that the orifice of some of these veins measures 3 mm. in diameter it was easy to determine by dissection that one was not dealing with ordinary depressions of the endocardium. They gave every appearance of being veins, but if we consider the development of the heart we must be very careful in making the statement that they actually are veins. The ventricular walls of the embryo consist of a sponge like network and into the trabeculae of this network the coronary capillaries extend. In places there is but a unicellular layer which separates the ventricular cavity from the coronary system. If there were actual intercommunication between the two we would be dealing with a sinusoid and the presence of venae minimae in the adult heart would indicate a persistence of this sinusoid. It is easy to conceive, however, of some of these trabeculae being pushed together by the growth of the ventricular cavity and not losing their endothelial covering in the course of development. These could, in the adult heart, easily simulate veins and their true nature can be determined only by injection of a fresh heart. In Bell's Anatomy (1812) we find the following statement, "Du Verney was so far engaged in this question (the presence of venae minimae), that having an opportunity of dissecting the heart of an elephant he tied up the coronary arteries and veins, washed and cleaned very thoroughly the cavities of the heart; and then tried, by squeezing, and all kinds of methods, to make that blood which was tied up in the coronary

arteries and veins exude upon the inner surface of the heart, but with no effect." In a previous paragraph they condemn the crude methods used by Vieussens, Thebesius and others who believe these openings to be openings of true veins.

The introduction of a needle in various parts of the septa atriorum and ventriculorum failed to reveal the presence of an os cordis, a fact which is surprising because its presence has always been associated with all large hearts, and a number of recent text-books state that it is present in the elephant's heart. This question has been the subject of a great deal of discussion ever since the time of Galen. Galen states that he found a bone in the heart of an elephant and kept it in his possession for a number of years. Moulin (1682), Blair (1708), and Perrault (1734) did not find it, attributing it to the youth of the animal they had under observation. Camper (1802), however, argued that in young animals one would at least find cartilage, as he did in a calf six weeks old, which he had dissected for the purpose of settling this question. In the elephant's heart he found neither bone nor cartilage. This seems to be the case in our specimen. It may well be that Galen had before him a sclerosis of a coronary artery. Mayer ('47) finds a "Knorpelstreif von 10 Linien am Ostium arteriosum des linken Ventrikels." The position he gives is too vague to comment upon. The size of the heart has evidently nothing to do with the presence or absence of an os cordis. My histological examinations have never failed to reveal bone tissue in the human and the dog's heart, and fibro-cartilage in smaller animals such as the rabbit and guinea pig. The lack of a bone in the heart of the elephant is due to poor development of the trigona fibrosa. Some of our leading text-books on human anatomy fail to recognize these trigones although they never fail to mention so unimportant a structure as the tuberculem venosum (Loweri) which is absent in the human heart. The trigona fibrosa (B.N.A.) are two masses of connective tissue which lie between the aorta and the orifice of the mitral valve. The left is near the periphery of the heart and the right in the triangle bounded by the aorta anteriorly and the mitral and tricuspid ring on the left and right side respectively. It is the

right trigonum which is the primary seat of the os cordis. A second os may develop in the left trigonum and may fuse with the right bone. In the elephant there is a slight shifting of position, due probably to the greater development of the conus arteriosus, so that the mitral ring does not lie on the same plane as the aorta but lower down and the right ring lies higher up. Posterior to the root of the aorta we have atrial musculature. This torsion was recognized by Miall and Greenwood who make the following statement:

> The right side of the heart is, so to speak, rotated upon the centre of the left; the right auricle being thrown to the dorsal surface of the heart, while the principal axis of the right side, passing directly through the auriculo-ventricular orifice, is inclined upwards, instead of lying nearly horizontal, as does that of the left side in the animal as it stands. The inferior face (anterior of man) gives no indication of this tilting of the right side, except that the right auricle is displaced; the two ventricles meet along a straight line and divide the lower surface nearly equally.

The cavity of the left ventricle is distinctly conical in shape but the lower two-thirds is very irregular owing to the large trabeculae and columnae carneae which are arranged mostly longitudinally to the axis of the heart. The upper third of the septal wall is comparatively smooth. There are two large papillary muscles controlling the mitral valve, the anterior measuring 5 cm. in diameter at its base and the posterior 2 cm. There are three distinct sets of cords or tendons attached to the mitral valve such as Kürschner ('44) has described for the human heart. The first set is fixed to the attached margin of the valve, the second to the middle, and the third to the free marginal edge.

The right ventricle is divided into two parts by the crista and trabecula supraventricularis.[4] The upper or anterior part, the conus arteriosus, ought rightly to be designated as the fifth

[4] In J. H. H. Bull., vol. 20, p. 172, 1909, I suggested the name trabecula supraventricularis to that portion of the crista which stretches across the cavity of the ventricle and corresponds to the moderator band of English authors and the bandelette ansiform of Poirier. The term moderator band was also used for all strands which stretched across the cavity of either ventricle, although in its restricted sense it applied only to the band which is constant in the sheep and pig.

chamber of the heart. A comparative study of the mammalian heart soon reveals the necessity of dropping the old division of the heart into four chambers, which may have had its didactic advantages, and of adding the conus as a fifth chamber. Both in its anatomy and physiology is the conus arteriosus somewhat distinct from the right ventricle and much confusion would be avoided by using the term right ventricle in its restricted sense. The right ventricle of the elephant has a rather small cavity, which at the lower third of the septum and the entire outer wall is traversed by large trabeculae and columnae carneae. There are three papillary muscles, but their peculiar arrangement can be understood only after a study of the conus has been made.

The conus arteriosus of the elephant seems to have the greatest capacity of any chamber of the heart, and differs from that of other hearts in that it has a large septal wall. We usually find the conus consisting of a very narrow septal and a very extensive free wall which give it its typical external shape from which its name is derived. In the elephant the increase of the septal wall is at the expense of the right ventricle whose septal area is correspondingly reduced. It is this difference of growth that has brought about the obliteration of the undefended space and the trigona fibrosa as before mentioned.

To understand the peculiarity of the position of the papillary muscles in the right ventricle of the elephant's heart it will be necessary to call attention to the condition usually found in other hearts. Detailed description will be found in papers by King ('37) and by Retzer ('09). The number of papillary muscles may vary from one to six and is inversely proportional to the size of the papillary muscles. The larger the anterior papillary muscles the fewer the number. King divided the mammalian hearts into four series according to the presence or absence of a moderator band. These series are typically represented by I, Rodent, Canine and Marsupial animals; II, Feline; III, Quadrumana and Human; IV, Ungulates. The trabeculae supraventricularis (moderator band) which always contains the right branch of the conductive-system (Reizleitungs system of Tawara) is found

constantly in series IV where the anterior or large papillary muscle that controls the greater part of the free cusps is situated on the free wall. In Quadrumana and Human this papillary muscle is situated about midway between the septal and free wall, its base resting upon a complex network of trabeculae. In series II the tendency is for this papillary muscle to arise from the septal wall, a condition which is almost invariable in series I. The number of papillary muscles seem to increase from series IV to series I. In all of these series the septal cusp is controlled either by numerous small papillary muscles and tendons arising from the septal wall itself or by a somewhat larger one situated near the posterior edge of the septum. The anterior papillary muscle is always the largest and is the most important for the control of the free cusps.

In the elephant we find the anterior papillary muscle controlling the septal leaflet while the two others control the free cusps. Their position is correspondingly on the septal and free walls. Such a condition is unknown to me nor does King mention it in his study of 300 hearts. The base of the anterior papillary muscle is situated on the septal wall immediately posterior to the crista supraventricularis and its apex points in the direction of the coronary sinus. It controls the entire septal cusp with the exception of its most anterior edge which is held by tendons that arise out of the septum. These tendons are constant structures in all mammalian hearts, and form one of the landmarks of division between ventricle and conus. In a few instances the author has found these tendons arising from a small papillary muscle whose fibers could be traced to a region near the pulmonary orifice. The muscle is of considerable size though not as large as the two others. Its position may be accounted for in the following manner. Usually the mesial cusp of the tricuspid has a broad base of attachment, many small tendons arising from the septum attaching to it throughout its width. The cusp in these instances cannot extend far into the cavity at the time of systole and the two other cusps are brought during contraction close enough to prevent regurgitation. In the elephant the size of the tricuspid orifice necessitates larger cusps (they measure 6.5 cm. in width)

and complete action of them all. We find correspondingly a septal cusp of a very narrow margin of attachment. The ventricle is however so short that it would be impossible for it to accommodate a papillary muscle long enough to control the mesial cusp, unless this muscles should act at an angle and thus reduce the necessary degree of contraction. Furthermore a large papillary muscle on the septum below the tricuspid orifice would be quite an obstacle and occlude a great part of the cavity. We find therefore the papillary muscle situated almost in the conus flattened agains the septal wall. This papillary muscle must therefore be considered as the homolog of the posterior papillary muscle in other animals, the change of its position being due to the unusual development of the conus and a corresponding decrease of the right ventricle. The other papillary muscles of equal size and slightly larger than the first one mentioned are situated on the free wall. The more anterior rests in part on the crista supraventricularis. They control the two free cusps. According to

ERRATA

The Anatomical Record, vol. 6, no. 3, March, 1912, page 83, tenth line from bottom, should read as follows:

The following measurements were taken: The diameter of the aorta 9.0 cm., thickness of wall 1.5 cm.; the diameter of the p. a. 6.5 cm., thickness of wall 1.0 cm.; the diameter of the cor. art. 1.0 cm., thickness of wall 0.2 cm.

valves. The following measurements were taken: The diameter of the aorta 9.0 mm., thickness of wall 1.5; the diameter of the p. a. 6.5 mm., thickness of wall 1.0; the diameter of the cor. art. 1.0 mm., thickness of wall 0.2. At the sinus of Valsava the thickness of the walls of both aorta and pulmonary artery is reduced by one-half. There are two coronary arteries, not one, as has been repeatedly quoted from Camper, and they are situated at the upper edge of the sinus of Valsalva. The vessels are joined together by loose areolar tissue and can easily be separated to their origin.

The atria are very small but this may be attributed to shrinkage. The walls are thin but in the auricles they are strengthened by many pectinate muscles. As stated above the veins were cut so near the heart that it is impossible to analyze the conditions thoroughly.

The following quotation is taken from Miall and Greenwood:

> Left Auricle—The entry of the pulmonary veins seems to vary. Vulpian and Philipeaux found two openings into the auricle—a small internal and a large external, which latter received three of the pulmonary veins. Dr. Watson describes four separate openings. In our dissection there was a large central orifice and a smaller one on each side—one internal and the other external; but the external vein was not altogether clear of the central one. A thin ridge upon the internal surface of the auricle separates the central from the internal orifice. The veins enter a thin and membranous sinus, which is slightly separated from the rest of the auricle by a prominent fleshy ridge. Part of the edge of this ridge forms the 'valvular structure' noted by Dr. Watson.

Very distinct in the left atrium is the semilunar fold which stands out like a crescentic valve. In its broadest portion it measures 1.5 cm. from the edge to its attachment. The fovea ovalis lies 3 cm. posterior to it, and is thin enough to be translucent.

The right atrium presents conditions that are very interesting from the view point of development. There are three vessels that empty into the right side of the heart, the inferior vena cava and the right and left superior venae cavae. The coronary veins empty into the left superior vena cava, where it is generally spoken of as the coronary sinus. The positions of entry of these vessels do not differ markedly from other hearts, except that there seems to be a narrower interatrial septum in the elephant so that the opening of the right superior vena cava lies nearer to the inferior than is usually the case.

The tuberculum intervenosum (Loweri) was looked for but, as usual, in vain. I have, so far, carefully examined the hearts of man, dog, cat, rabbit, guinea pig, bear, sheep, rat, and opossum, which is a sufficiently representative group to say with absolute assurance that there is no such thing as a *tuberculum* intervenosum. By a tuberculum we mean a tuberlike or knoblike pro-

jection. It is in this sense that I deny its presence, and it was, therefore, a great satisfaction to find the following quotation from Haller, "Id tuberculum cupide receptum est, ut fere fit, ab iis scriptoribus quibus occasio ad propria experimenta nulla est, deinde etiam ab iis qui tandem omnino in corporibus humanis dissecandis se exercuerunt." It would be too much of a digression at this point to go into the details of development but suffice it to say that the tuberculum intervenosum never does exist at any stage of development, and Pohlman ('09) has shown that there is no physiological necessity for it. In the developing heart it can be clearly seen that the tuberculum intervenosum is nothing but a ridge which is the continuation of the limbus fossae ovalis, and it depends upon the size and position of the limbus whether or not there is any 'crista' intervenosum on the upper outer wall. In the elephant's heart the superior vena cava is separated from the inferior by the limbus fossae ovalis, which lies immediately posterior to it and slightly bulges the septal wall inward, i.e., into the cavity of the right atrium, and by the fossa ovalis itself. The posterior margin of the fossa ovalis lies really within the inferior vena cava.

It is unfortunate that the specimen is so imperfect that the extent of the valves cannot be definitely determined. Miall and Greenwood make the following statement:

> A sigmoid valve passes from the external side of the right anterior cava, adjacent to the appendix, curves round the ventral side of the orifice, and is then continued as a long membranous ridge of slight projection to the basal or anterior side of the posterior cava; it then crosses that opening on its ventral margin, becoming somewhat more prominent, and serving as a proper valve to the posterior cava; finally, it gradually disappears along the base of attachment of the Eustachian valve. This agrees tolerably well with the description of Vulpian and Philipeaux; and with Dr. Watson's figure, though in the text of his description he says that the valve passed round the upper margin of the right anterior cava. A large Eustachian valve separates the posterior from the left anterior cava. The great coronary vein opens into the left anterior cava under cover of a pectinated muscle.

What remains of my specimen bears out the description as regards the position of the valve of the superior vena cava and it seems likely that it is continuous with the valve of the inferior

vena cava, but the statement that "it gradually disappears along the base of attachment of the Eustachian valve" demands some explanation.

This entire valve and fold is the persistent right venous valve which in the young foetus stretches as a crescentic fold from the right side of the superior vena cava along the upper wall of the atrium, thence along the right side of the opening of the inferior vena cava to disappear in the septum intermedium of His. As the coronary sinus develops the lower end of this valve forms the valve of the coronary sinus (Thebesii). The persistent part of the valve near the orifice of the inferior vena cava becomes the Eustachian valve. Owen states "The right auricle in the Rhinoceros, as in most Ungulates, has but one precaval orifice, and shows no valve at the termination of either the postcaval or coronary veins; the contrast presented by the elephant, in this respect, is significant." Unfortunately Owen fails to define the contrast and one can but assume that the elephant has two precaval orifices and valves guarding all the openings. Cuvier ('39) says (Meckel's translation) p. 41 "Beim Elephanten is diese (Eustachische) Klappe spiral förmig gewunden und geht längs der oberen Wand in das hintere und linke Ende einer anderen breiten und halbmondförmigen Klappe über, welche die Oeffnung der vorderen rechten Hohlvene von der Höhle des öhrformigen Anhanges scheidet." Wolff, according to Meckel in Cuvier's Vorlesungen, IV, p. 36, and Meckel himself have found instances where in absence of a Thebesian valve the Eustachian extended so far down so as to cover the opening of the coronary vein. From this it is evident that these older authors called the Eustachian valve a different structure than is understood by that name today. Search through the literature revealed the fact that the Eustachian valve was formerly called valvula forminis ovalis anterior (Bock, ('50), Soemmering ('41). That the Thebesian valve is but a continuation of the lower end of the Eustachian is a discovery that we owe to His and Born.

What then did Miall and Greenwood mean by "A large Eustachian valve separates the posterior from the left anterior cava?"

The valve they have reference to is a very prominent structure in our specimen. It is a fleshy valve, with a straight edge, about 4 cm. in length and separates the opening of the inferior posterior vena cava from the left anterior just as is stated by these authors. It can readily be seen that this is the homolog of the fold that is found in all the animals that have a persistent left superior vena cava. Marshall ('50) was the first to call attention to the fact that the Thebesian valve is always absent in the hearts of those animals which have a persistent left superior vena cava or left azygos emptying into the heart. Watson thinks that the fold that he found in the elephants heart is a Thebesian valve and concludes therefore that it forms a marked deviation from the rule as formulated by Marshall. Born ('89) describes this fold in the rabbit embryo and calls attention to the fact that it is not to be considered a Thebesian valve. A study of the developing heart of the pig reveals the fact that the lower end of the right venous valve aborts while in the human it grows over this same fold which is so prominent in the elephant, pig, sheep, rabbit and other animals. It is really not a valve but a fold that has grown in between the sinus (left superior vena cava) and the inferior vena cava. This fold has not been recognized by previous authors and will be discussed at length in another article.

Although the terminology differs we find most authors agreeing that the valve of the posterior vena cava is continuous with that of the anterior. These valves are the remains of the right venous valve of the embryo. It is interesting to note in this connection that the crista terminalis from which the pectinate muscles take their origin is present in the elephant but about 2 cm. to the right and below the valve guarding the right superior vena cava. It needs a further study to determine whether the crista terminalis really is the boundary between the primitive sinus and the right atrium. This idea has its foundation upon the theory that the right venous valve terminates on the crista terminalis, a theory which is not substantiated by the condition in the elephant's heart just described nor by any stage of development of the embryo pig's heart.

The coronary veins, two in number, empty into the left superior vena cava, their openings protected by valves. The pectinate muscle that Miall and Greenwood mentioned is also present.

The conductive system is visible in the right ventricle as a light colored strand about 3 mm. in diameter and lying along the upper border of the crista and trabecula supraventricularis. Its course is superficial in the ventricle but at the atrio-ventricular junction and in the atrium itself it lies embedded in musculature. Here also it has a different appearance and character. While in the ventricle it is a glistening white strand when dissected out and elastic, in the atrium it is dark red, tough and irregular in outline. This is evidently the nodal point (Knoten of Tawara). It measures about 1.5 cm. in diameter and 0.5 cm. in thickness and lies 2 cm. below the edge of the sinus fold and 4 cm. from the junction of mesial and anterior leaflet of the tricuspid. The left branch seemed at first to be wanting but after painstaking search its presence was established. As previously stated there are no Purkinje fibers visible in the ventricle and the inverted V-shaped strand of the left branch of the conductive system that is so constant in all other mammals as far as we know, is not visible nor did dissection reveal it. The left branch leaves the nodal point to the left of the right branch deeply embedded in the myocardium and soon after seems to divide into two branches whose course in the ventricle could not be established without destruction of the specimen. At the level of the aortic cusp it was deeply embedded in the ventricular musculature.

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VARIATIONS IN THE CHARACTER OF GROWTH IN TISSUE CULTURES[1]

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NINE FIGURES

The character of the growth in tissue cultures varies primarily with the kind of tissue used. Differences are often observable in the growths from corresponding organs of animals of different species. For example, cultures of chick embryo spleen and of rat spleen may be differentiated by the shape and size of the cells and by the behavior of the fat droplets in the cells of the older cultures. The growths even of certain tissues of so closely related species as rats and mice are under some conditions distinguishable. The several tissues of the same animal exhibit still more definite cultural differences. The complex character of the organs of birds and mammals, however, makes the isolation of special tissues in culture media difficult, but that it is often possible by careful observation of fresh and stained preparations to differentiate the component tissues is shown by a summary of the work done bearing on this point. Burrows[2] recognized in sixty-hour chick embryos the growth of nerve fibers and mesenchymal tissues. Carrel and Burrows[3] described a tubular growth of the kidney epithelium of dogs and cats, and a tubular and sheet-like growth of thyroid parenchyma. In each case the parenchymal growth presented a sharp contrast to that of the

[1] Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

[2] M. T. Burrows. Jour. Exp. Zool., vol. 10, p. 63, 1911.

[3] A. Carrel and M. T. Burrows. Jour. Amer. Med. Assn., vol. 55, p. 1379, 1910; also Jour. Exp. Med., vol. 13, p. 416, 1911.

accompanying connective tissue. Fleisher and Loeb[4] made similar observations in cultures of the kidney of rabbits and guinea pigs, and also recorded growth of the epithelial covering of the ovary distinguishable from that of the other elements. Lewis and Lewis[5] in their studies on the growth of chick embryo tissues in artificial media, agar, buillon and salt solutions, observed, in addition to radiating and reticular formations, common to growths from practically all organs, a definite growth of sympathetic nerve fibers, and a characteristic sheet-like growth from pieces of intestine, interpreted as peritoneal mesothelium. Fig. 1 shows such a membrane from a three-day culture in plasma. The morphology of the cells composing the sheet favors the interpretation given by the authors.[6]

Dr. Hanes and I described elsewhere[7] a striking contrast in the character of the growth obtained with the epithelial tumors and with the connective tissue tumors of rats and mice, the one being sheet-like and alveolar in type, the other, radiating with strings of irregularly shaped cells. We suggested that these two types might represent in a general way the character of the growth in vitro of the corresponding normal tissues. Some of the observations quoted above on the cultivation of mammalian organs, together with our recent experiences with certain organs (spleen, bone marrow, and ovary) of rats, mice and guinea pigs, and several tissues of the chick embryo, (skin, intestine, heart, liver and spleen) have supported our suggestion. That is, growths from organs which do not contain epithelial structures have never shown a true sheet-like character, while in cultures of skin and intestine this type of growth has occurred rather regularly. The outgrowth in liver cultures has consisted entirely of connective tissue. Groups of cells in close apposition growing along the under surface of the cover glass have been seen not infrequently in cultures of chick embryo heart and rat spleen,

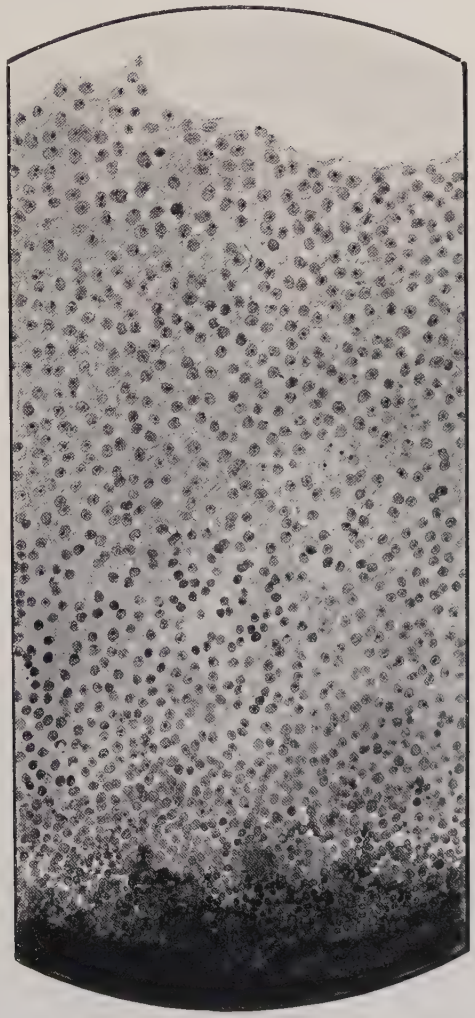
[4] M. S. Fleisher and L. Loeb. Proc. Soc. Exp. Biol. and Med., vol. 8, p. 133, 1911

[5] Margaret R. Lewis and W. H. Lewis. Anat. Rec., vol. 5, p. 277, 1911.

[6] In a more recent paper (Anat. Rec. 1912, No. 4,) the authors are inclined to interpret these sheets of cells as outgrowths of intestinal mucosa.

[7] R. A. Lambert and F. M. Hanes. Jour. Exp. Med., vol. 13, p. 495, 1911.

but definite sheets of cells of the type observed in the growth of epithelial tissues have not been obtained.



All drawings were made from preparations stained with Weigert's iron hematoxylin after formalin fixation.

Fig. 1 Three-day culture of chick embryo intestine, showing formation of a wide sheet of cells. A majority of the cells contain a single large fat globule. Cell margins are quite distinct.

In the following paragraphs the influence of the character of the culture medium, mechanical factors, addition of foreign bodies, and temperature on the growth of tissues in vitro will be discussed.

GROWTH OF TISSUES IN HETEROLOGOUS PLASMA

In another paper[8] the suitability of different kinds of alien plasma as culture media for rat and mouse tissues was discussed and reference made to variations in the morphology of the cells in the different media. The cells of the malignant connective tissue tumors (sarcomata) showed this change most distinctly. For example the growths in rat plasma, pigeon plasma and human plasma, apart from the rate and extent, presented characteristics that rendered them readily distinguishable. In human plasma, disappearance of the fibrin with a wandering out of the cells over the cover glass and giant cell formation, and in pigeon plasma the regular radial spreading of uniformly large clear spindle cells connected by processes, gave appearances altogether different from the diffuse radial spreading of triangular and irregularly shaped cells in homologous plasma. Studies with the connective tissue of chick embryos have demonstrated, in like manner, a decided effect on the rate and character of growth from the use of foreign plasmas as culture media, but the variations for the different media were found to be not so characteristic as in the tumor cultures. Pieces of chick embryo heart in human, rabbit and rat plasmas gave rise to feeble growths of long, slender, granular cells ending in delicate processes. In human plasma there were sometimes seen, in addition to these spindle cells, large coarsely granular cells with ragged outlines moving out on the cover glass. Fig. 3 shows the appearance of the cells and the extent of growth in a four-day culture in rat plasma. The epithelial tumors studied did not show striking variations under the similarly modified conditions, except in human plasma where numerous multinucleated cells were formed. This can be explained by the fact that epithelial cells tend to remain adherent in

[8] Jour. Exp. Med., vol. 14, p. 129, 1911.

sheets or in groups without individual free protoplasmic borders thus making variations in cell outline less likely to take place.

These morphological variations in heterologous plasma are not to be attributed altogether to chemical and biological differences in the media per se. It is obvious that certain physical differences may account to some extent for some of the variations. For instance, the disappearance of the fibrin in clots of human plasma containing rat tissue introduces an important mechanical factor whose effect on growth will be discussed in a subsequent paragraph. The duration of growth, however in foreign plasma as determined by observations on single cultures and by the effect of transferring the pieces of tissue to fresh plasma has shown that biological differences may exert a marked influence on the length of life of cells in vitro.

MECHANICAL FACTORS INCLUDING THE ADDITION OF FOREIGN BODIES

The mechanical factors influencing the growth of tissues in vitro were discussed by Harrison[9] in his earlier reports of cultures in frog's lymph. More recently in a paper on 'Stereotropism in Embryonic Cells'[10] he demonstrated conclusively that for the outgrowth of cells in cultures some kind of mechanical support is necessary. This support may be supplied by the fibrin in clotted plasma or lymph, the lower surface of the cover glass in fluid media, or by some added foreign framework such as spider webs. For the cover glass to act as a support it was shown that the pieces of tissue must be adherent to it. That is, in cultures in which the tissue floated free in the hanging drop of fluid no outgrowth occurred.

It is easy to see that with tissues giving a radiating growth of independent cells the density of the outgrowth may be modified by the thickness of the drop of clotted medium, the thicker drop giving a denser growth, and that the general effect of cells growing in one plane as is the case in fluid media, is different from that in

[9] R. G. Harrison. Jour. Exp. Zool., vol. 9, p. 787, 1910; also,
[10] Science, vol. 34, p. 257, 1911.

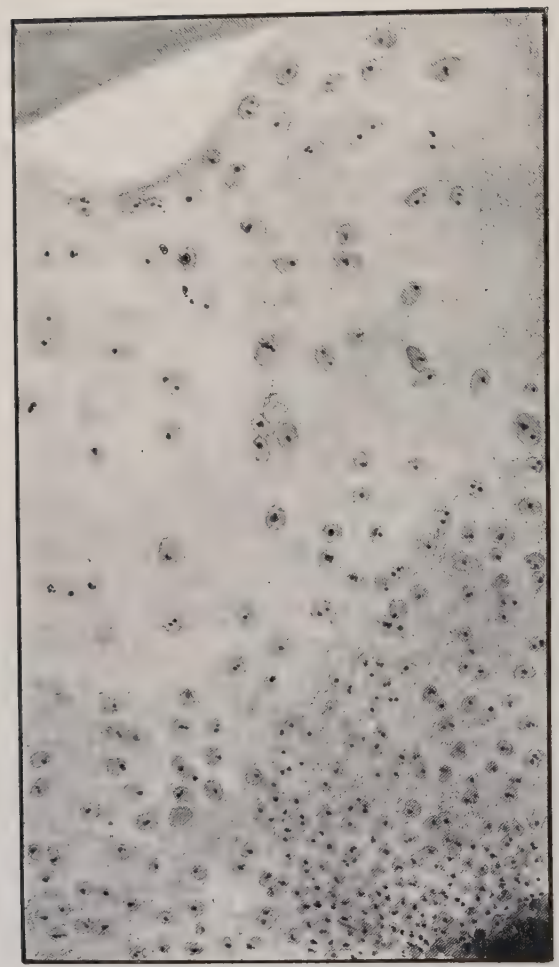


Fig. 2 Four-day culture of chick embryo skin showing a sheet-like spreading with marked flattening of the cells. Cell boundaries are not visible.

plasma where cells wander out at various levels. We have further observed in certain tissues a difference in the morphology of the cells under the two conditions, the cover glass cells tending to be much more flattened. This phenomenon is well illustrated in cultures of rat spleen where cells wandering through the clot and along the cover glass may be seen in the same preparation.

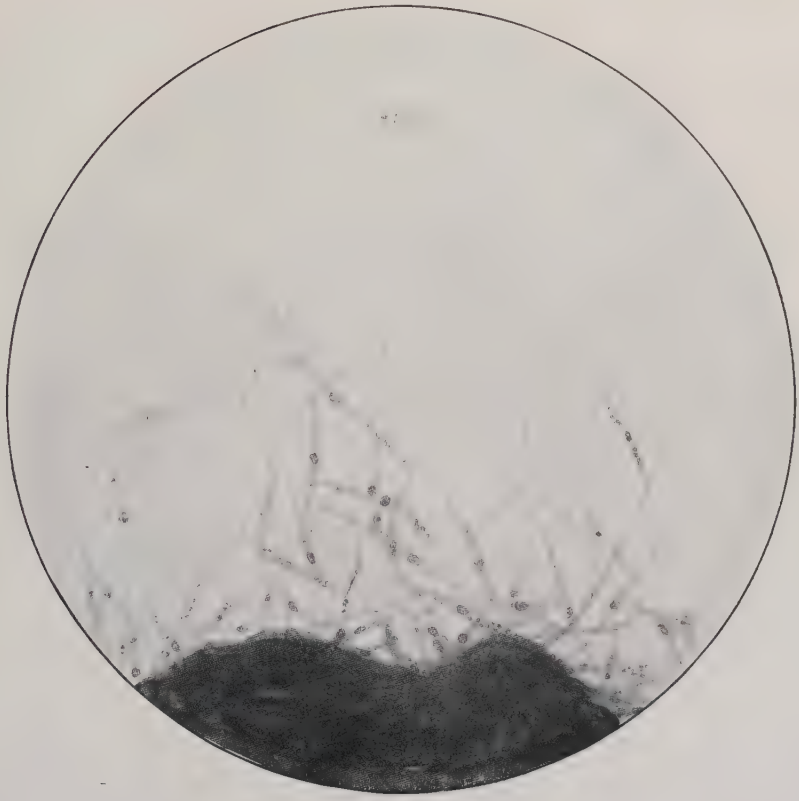


Fig. 3 Four-day culture of chick embryo heart in rat plasma showing feeble growth of long granular spindle cells with delicate processes. Compare with fig. 4.

The lateral dimensions of the cover glass cells, involving both nucleus and cytoplasm, are much greater, but there is an accompanying diminution in the thickness of the cells. In observing cultures of rat spleen in human plasma, where on account of the disappearance of the fibrin only cover glass cells are found, cells have been seen to change from thick rounded forms to large flat cells with a delicate filmy cytoplasm. Connective tissue cells seem to be less labile and show only a moderate increase in width when growing on the cover glass. In the sheets of epithelial cells in skin and tumor cultures a marked flattening of the cells is often seen (fig. 2). It is possible that tension produced by contraction

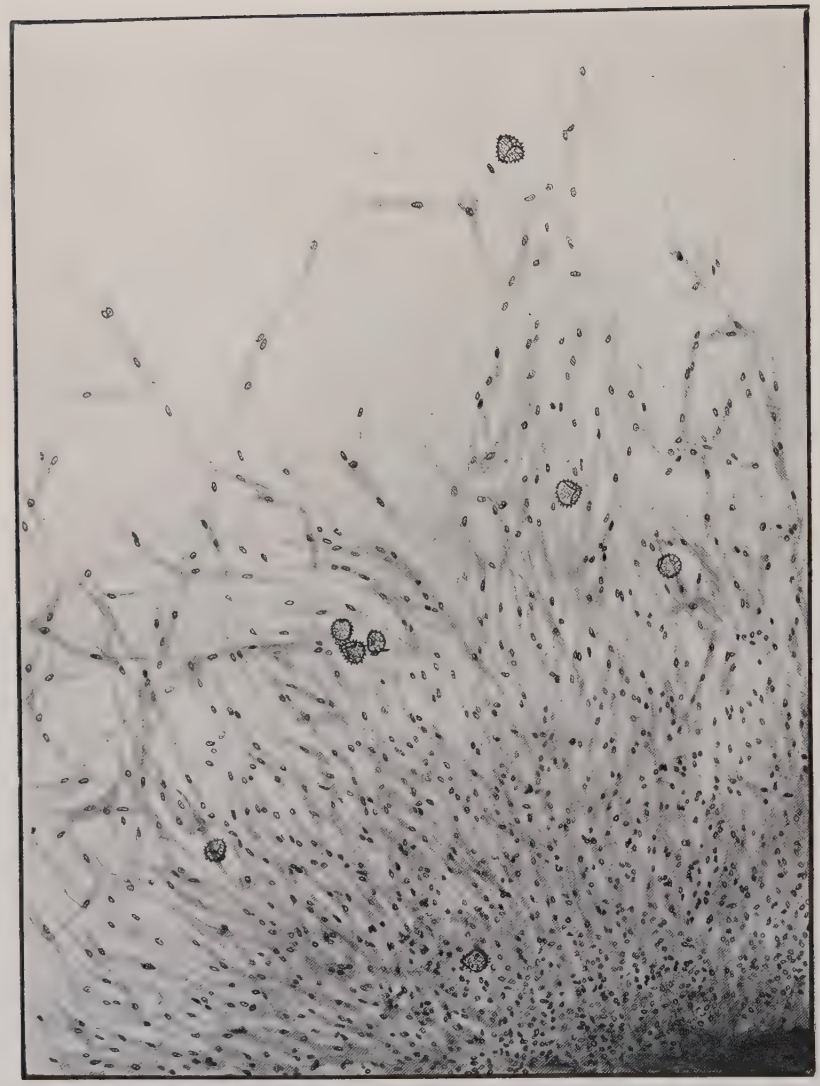


Fig. 4 Four-day culture of chick embryo heart, showing diffuse connective tissue growth with no tendency to giant cell formation about foreign bodies (lycopodium spores).

of the fibrin to which the border of the sheet is attached may exert an influence in producing this appearance. Detachment of the sheet from the fibrin occurs quite often in tumor preparations, resulting in a retraction of the cells into a mass about the original piece of tissue, or toward that part of the clot which has held. In such cases fenestra are left into which cells may subsequently wander along the cover glass. Harrison described changes in the shape of individual cells produced by contraction of the fibrin in coagulated lymph, and showed further that they might be moved for a considerable distance by this means.

The effect of the thickness of the drop of fluid on the morphology of the cells on the cover glass was observed in a few preparations of spleen and bone marrow in which the drop accidentally touched the side of the slike cavity after some cell wandering had taken place, leaving an extremely thin film of fluid over the cells. A flattening out of the cells to a remarkable degree followed. Dr. Hanes obtained exquisite granular pictures from such preparations after Altmann's fixative and stain, the cell granules being widely scattered and well defined.

Giant cells

The formation of giant cells will be discussed in this connection because we feel convinced that certain mechanical factors just referred to are concerned in their production. The fact that certain types of giant cells are situated so constantly on the cover glass suggests at least a causal relationship. Giant cells of several types are observed in the cultivation of rat and chick tissues.

1. Cells with two or three nuclei and abundant cytoplasm, four to five times the size of ordinary cells, are encountered very frequently in tumor cultures. The largest number have been seen in the cultures of mouse tumor in human plasma. They are usually situated on the cover glass.

2. Cells with three to one hundred nuclei, generally arranged centrally, cytoplasm presenting bulbous and irregular processes, are seen in large number in cultures of rat spleen in human plasma (figs. 5 and 6). They vary from 100 to 900 micra in diameter,

Fig. 5 Giant cell from a six-day culture of chick rat spleen in human plasma, showing large bulbous process, and an adjacent mononuclear cell for comparison.

and are always spread out in a thin sheet on the cover glass. In studying the process of their formation difficulties were encountered. In the first place they are formed as a rule in the zone of attachment of the piece of tissue to the cover glass, and are consequently not distinctly visible until they wander out. In the hope of settling the question as to origin from a single cell or from fusion of a number of small cells, single cells and aggregations of cells of ordinary size have been watched for several days in cultures kept under continuous observation in a warm wooden microscope box. A transformation of relatively small round bodies to large flat multinucleated giant cells gave at first the impression of development from single cells. Subsequent study, however, of these round bodies showed that they were often quite thick and not unquestionably mononucleated. Moreover, the transformation of a typical multinucleated giant cell into a round granular mass, and then a return to the original form was observed in a fresh preparation. The diameter of this giant cell was several times greater in the second, or flattened out stage, than during the first period.

The formation of giant cells of this type from fusion of aggregated cells has not been seen, although such groups have been carefully watched for four or five days. Stained preparations often show appearances indicating a process of fusion but continuous observation of fresh cultures, where appearances of this kind are followed by a separation of the cells throws doubt on the interpretation suggested. Indeed, cells moving over the cover glass are often seen passing over one another. Round inactive looking cells are commonly observed attached to giant cells, and frequently become incorporated in their cytoplasm (fig. 6). Foreign particles are also taken up phagocytically.

3. Giant cells with ten to one hundred or more nuclei have been observed in cultures of chick embryo spleen and intestine. These are sometimes in the form of large plasmodial sheets with nuclei scattered irregularly throughout. Others present a more or less central massing of the nuclei. They appear as a rule thinly spread out but are not always attached to the cover glass. The mechanism of their formation has not been studied.

4. Giant cells may be formed about foreign bodies. These have been produced at will by adding lycopodium spores to cultures of chick embryo spleen. Under favorable conditions, a large proportion of the spores are surrounded during the first two days by the active wandering cells (fig. 7), and many of the cell masses so formed subsequently become transformed into giant cells (fig. 8). These giant cells differ as a rule quite markedly from those described above. They are usually very thick, the enclosed spores being often quite invisible, and show little tendency to spread themselves on the cover glass. Some of them however, present pseudopodia and are able apparently to alter their spatial relations. Giant cells are formed about the lycopodium spores attached to the original pieces of tissue in the culture as well as about those in the zone of wandering cells. The former, though sometimes visible in the fresh preparations as dense rings about the spores (which are made more easily recognizable by previously staining with neutral red or methylene blue), are studied best in stained paraffin sections. They seem to be formed somewhat more slowly than those in the zone of wandering cells. Our best preparations were obtained from eight and ten day cultures. The latter group of giant cells, on the other hand, often show pyknotic nuclei after four to five days. Early accumulation of fat droplets occurs regularly and occasionally large vacuoles are seen (fig. 8). In order to investigate further the kind of cells concerned in the formation of these giant cells, spores were added in the same way to cultures of chick embryo heart where the outgrowth consists entirely of connective tissue. No tendency to giant cell formation was ever observed.

These observations are of more than passing interest because of their bearing on the process of formation of foreign body giant cells in the body, particularly on the question as to the kind of cells concerned in the process. The designation 'wandering cells' used in describing the outgrowth in spleen cultures, may, and probably does include cells of several types—leucocytes, endothelial cells. It was recognized too that connective tissue cells might under some conditions resemble wandering cells. The studies

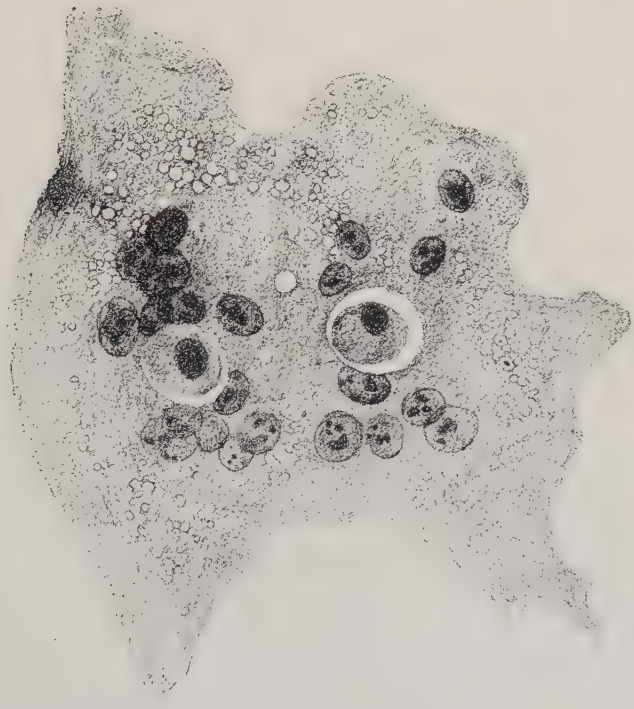


Fig. 6 Giant cell from a six-day culture of rat spleen in human plasma, showing phagocytic inclusion of two large mononuclear cells.

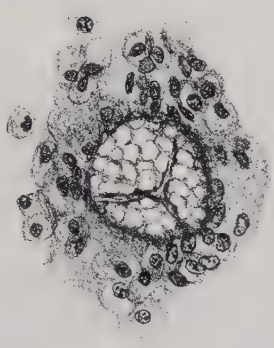


Fig. 7 Massing of wandering cells about a lycopodium spore in a four-day culture of chick embryo spleen.

with the abundant connective tissue in heart cultures leaves little doubt, however, as to the passive rôle played by these cells. Definite conclusions with regard to the formation of foreign body giant cells in general are, of course, not to be drawn from these

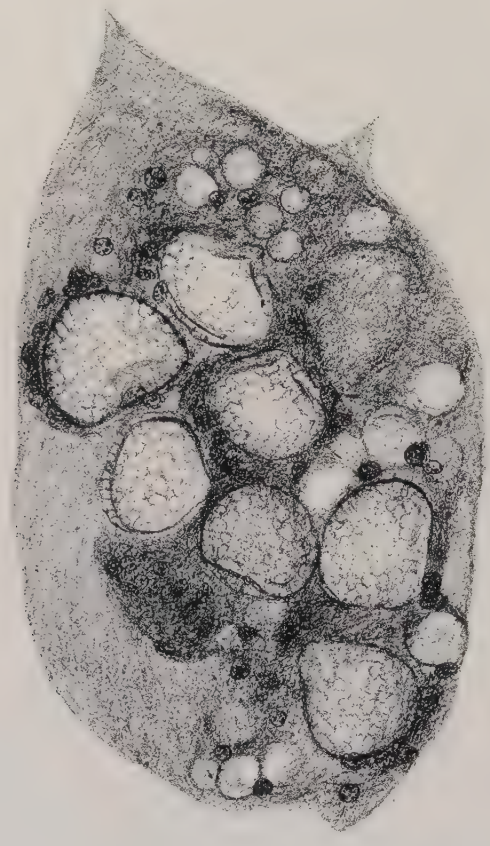


Fig. 8 Large giant cell formed about seven lycopodium spores, from a five-day culture of chick embryo spleen. The thickness of the cell prevented satisfactory differentiation of nuclei.

studies. The work throws light on the particular problem investigated—the reaction of cells in tissue cultures to foreign bodies—, and furthermore demonstrates the value of the method as a means of attacking similar problems.

Of the four types of giant cells described the process of formation of only the last variety—foreign body giant cells—is entirely clear. The multinucleated tumor cells probably represent the effect of division of the nucleus without division of the protoplasm,

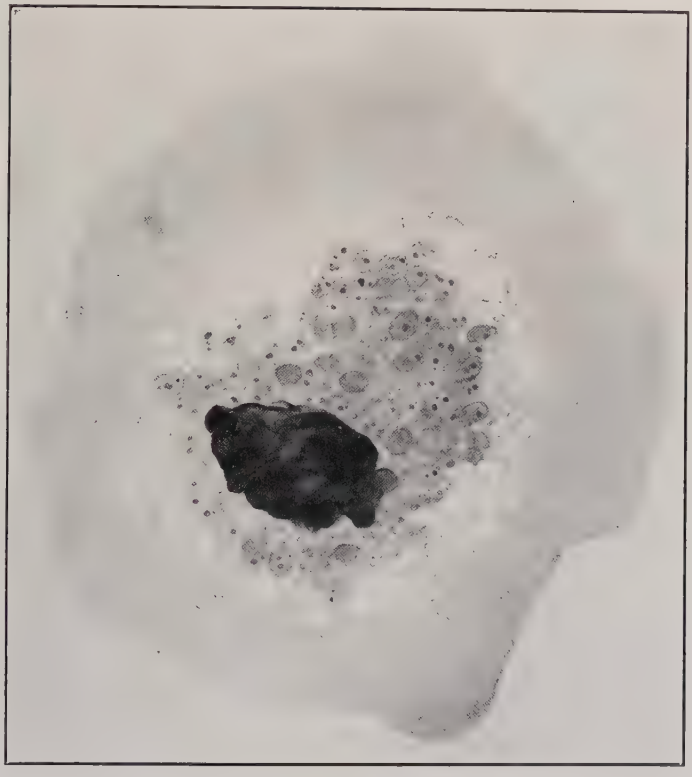


Fig. 9 Giant cell containing a mass of débris, from a five-day culture of chick embryo spleen.

a common occurrence in the animal body. The formation of the large multinucleated giant cells in cultures of rat spleen about the area of attachment of the piece of tissue to the cover glass suggests at least that mechanical agencies are concerned in the process.

THE EFFECT OF TEMPERATURE ON THE GROWTH OF EMBRYONIC TISSUES

Pieces of heart from eight to twenty-day chick embryos have been used in these experiments. It has been found that the range of temperature at which growth will take place is surprisingly wide. Cultures left at laboratory temperature which varies from 21° to 27° showed a slow steady growth which in some instances continued longer than in those at 38°, but were never comparable in the rate and extent of growth to the incubated cultures. Pieces of heart from nine and ten day embryos seemed to beat longer and more regularly at 29° than at 38°. A number of the preparations at room temperature (26°) continued beating for several days, some for six and seven days. One of these was placed on the third day in the ice box at −1° for twelve hours. Forty-five minutes after being returned to room temperature (26°) beating was resumed with practically the same rhythm as before. The outgrowth of connective tissue cells also showed no change. In previous experiments it had been found that forty-eight hours in the ice box (−4°) did not influence in any way the subsequent behavior of pieces of heart in incubated cultures. The effects of freezing and of subjection to very low temperatures have also been studied. The detailed experiments will be given in another paper. Some of the results may, however, be briefly recorded at this time. Small pieces of heart are frozen at about −10°. Freezing at this temperature five to ten minutes modifies very slightly subsequent growth at 38°. A sparse connective tissue outgrowth may take place after two to six hours freezing. That the heart muscle also survives is shown by the rhythmical contractions of some of the pieces. Freezing two minutes at −18° is apparently not harmful, but fifteen minutes exposure prevents any later activity of the tissue. The same cells may be killed in less than a minute by means of a CO_2 freezing apparatus, provided the freezing plate is first allowed to become thoroughly chilled. The temperature secured in this way was not accurately determined.

The upper temperature limit was found to be more definite. At 44° there was a diffuse outgrowth lasting several days, some-

what less extensive, however, than in the cultures at 38°. At 46° no growth took place but subjecting the cultures to this temperature for forty-five minutes did not serve to prevent the usual growth at 38°. Higher temperatures proved more injurious. Cultures heated to 50° for forty-five minutes showed subsequently outgrowths of only occasional cells. Those placed at 55° for twenty minutes remained quite inactive.

It is of interest to note that the connective tissue cells in the cultures at the different temperatures showed no morphological variations. The few cells which survived heating to 50° for forty-five minutes were not different in appearance, either in the fresh state, or in the stained preparations, from cells in cultures kept at 38°.

SUMMARY

1. Certain specialized tissues of mammals and of chick embryos present characteristic types of growth in culture media.

2. Variations in the character of the culture medium through the use of the different kinds of alien plasma influence the morphology of the cells in the cultivated tissues, particularly of the connective tissue rat tumors. The connective tissue of the chick embryo and the epithelial mouse tumors are influenced to a less extent.

3. Some of the mechanical factors influencing the character of the growth of tissues in culture media are: depth and consistence of the hanging drop, relation of the cells to the cover glass and foreign bodies, and contraction of the fibrin in the clotted plasma or lymph, producing tension on attached cells.

4. Mechanical factors are concerned in the production of some of the giant cells observed in tissue cultures. Those obtained in the cultivation of mouse, rat and chick tissues are of several types: (1) Cells with two to three nuclei and abundant cytoplasm encountered most often in tumor cultures. (2) Cells with three to a hundred nuclei centrally situated, commonly observed in the cultivation of rat spleen and bone marrow, and obtained in largest number when human plasma is used as a culture medium. They are actively phagocytic for dead cells and foreign bodies.

They are formed chiefly in the area of attachment of the piece of tissue to the cover glass, and are always spread out on the cover glass over which they move very readily. (3) Cells with ten to a hundred or more nuclei arranged either in the center or scattered irregularly through the cytoplasm, observed in cultures of chick embryo spleen and intestine. (4) Foreign body giant cells formed in cultures of chick embryo spleen upon the addition of lycopodium spores. They are produced through the fusion of wandering cells which mass themselves about the foreign particles. The addition of lycopodium to cultures of chick embryo heart where the growth consists entirely of connective tissue does not lead to the formation of foreign body giant cells.

5. A wide range of temperature is compatible with the growth in vitro of chick embryo tissues. Cultures at temperatures ranging from 27° to 44° differed in rate of growth, the optimum temperature being around that of the body, but differences in the morphology of the outgrowing cells were not observed. Pieces of heart remained beating for seven days at room temperature (21°–27°) and showed a slow connective tissue growth. Pieces of heart subjected to a temperature of 50° for forty-five minutes and subsequently incubated showed only an occasional outgrowing cell. No activity followed heating to 55° for twenty minutes. Pieces of heart placed in the ice box at −4° for forty-eight hours did not behave differently in cultures from untreated heart.

The effect of lower temperatures associated with freezing varied with the time of exposure and the temperature reached.

GROWTH OF TISSUES IN CULTURE MEDIA AND ITS SIGNIFICANCE FOR THE ANALYSIS OF GROWTH PHENOMENA[1]

LEO LOEB

From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis

Conditions occurring in nature may guide us in devising experimental methods for the analysis of natural phenomena. Experimental analysis must start from and remain in close contact with natural developments in living organisms. That tissues may grow in culture media outside and inside the living body was apparent from the relative ease with which as I observed in my early studies on regeneration, transplanted epithelium almost altogether or altogether separated from the neighboring host tissue may grow in blood clots or coagulated lymph. Under these conditions in which the supply of food material must have been minimal, the tissues could grow in the coagulum and migrate quite independently of any contact with other tissues.[2]

This suggested to me at that time that it should be possible to cultivate tissues on solid culture media in the test-tube as well as within the body, the latter serving as a natural incubator and that it should be thus possible to observe the behavior of different kinds of cells independently of each other. Tissue cells should in principle be accessible to indefinite propagation in a similar manner as bacteria. And I may add here that a few years later I produced experimental evidence making it very probable that certain somatic cells are at least potentially immortal.[3]

[1] My recent experiments were carried out in collaboration with Dr. Moyer S. Fleisher and some of them with Dr. Marsh Pitzman of St. Louis. Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

[2] Über Regeneration d. Epithels Arch. F. Entw. Mech. Bd. 6, 1898.

[3] Virchow's Archiv, 1891.

Experiments were accordingly undertaken by me in vitro—using coagulated bloodserum, bloodclot and agar as culture media, as well as in vivo.[4]

Conditions did not make it possible for me at that time to carry the first named experiments sufficiently far. The latter series of experiments, however, I carried to a definite conclusion. The investigations upon which I shall report very briefly at this occasion, are founded on the same principle as my earlier experiments, a continuation of which they represent, as far as their theoretical basis, the specific problems and the methods used are concerned.

The conditions which determine the fate of tissues in culture media are very complex. It will need much future work to make the analysis complete. Our investigations permit us to draw the following preliminary conclusions:

We have to distinguish between three different states in the equilibrium of tissues, first: the life of the tissues as it is principally determined by its morphological characters, leaving out of consideration at present the exercise of the specific functional activity. Secondly: tissue growth as it is determined by mitotic and amitotic division of the cells, by the size and characteristic staining of the nucleus and cytoplasm. Thirdly: the movements of the tissues depending on the locomotion of the constituent cells. These conditions constitute equilibria of a very labile character, and the same holds good of a fourth state, that of ordinary tissue death, which represents likewise a labile state of equilibrium, inasmuch as it is at once followed by processes of autolysis.

We first may ask, whether these various states of tissues and cells depend upon the same internal and external conditions or whether there are certain factors of specific significance for each one of them. Life and growth of the tissues are influenced in a similar direction at least by some factors, while tissue movements depend on a special condition.

[4] Chicago, 1897, Arch. F. Entw. Mech. Bd. 13, 1902; and Journal Med. Research, vol. 8, 1902.

In the course of ordinary tissue transplantation, the center of the transplanted piece becomes necrotic. The same holds good, if we place a piece of tissue in culture medium. We may assume that this is due to the interruption of the circulation which carries to the tissues various substances, oxygen as well as dissolved food material. Which of these two substances, the lack of which is responsible for tissue death and for the injurious influence which the products of autolysis in all probability exert upon the peripheral living cells is a question. Our experiments show that complete lack of oxygen not only prevents the growth of tissues, but causes their complete death. Anaerobic life seems to be impossible for mammalian tissues; this applies to tissues of normal organs as well as to tumors. Anaerobiosis can be produced in these experiments in an analogous manner as in the case of bacterial cultures. Various methods give the same result. In this case we see a parallelism between the effect of an external factor upon growth and life. Both states are equally impossible without oxygen.

In experiments on the other hand in which we increase the oxygen in the atmosphere surrounding the piece of tissue, we found both the life of the tissues and the growth phenomena increased. This was noticeable, although not in a very pronounced degree, in our first experiments, in which we merely filled the jar in which the test-tubes containing the pieces of tissue and culture media had been placed with oxygen and prevented the escape of the enclosed gas.[5]

It was more marked in our later experiments, in which we kept a constant though slow current of oxygen passing through the jar. In these experiments in which we used tumor (carcinoma of the mouse) and kidney of the rabbit, we found the peripheral zone of living tissue distinctly enlarged under the influence of the oxygen and furthermore in certain cases the number of mitoses in the kidney which was especially examined from this point of view was noticeably increased. In these latter experiments there was as yet present one possible source of error. It might be that through the current of oxygen passing very slowly through the jar

[5] Biochem. Zeitschrift, October, 1911. Science, September 29, 1911.

the evaporation of water is accelerated in the test-tubes standing in the jar. This factor has to be taken into consideration, notwithstanding the fact that the test-tubes are closed by cotton. Inasmuch, however, as we took precautions to avoid evaporation and especially inasmuch as even after previous addition of strongly hypotonic fluid to the culture medium, the same results were obtained, it is not probable that an increase in osmotic pressure in the surrounding medium is responsible for the effects that we observed. Furthermore it would be difficult to understand how such an increase in osmotic pressure should be capable of increasing the number of mitoses in the tissue. We are, however, at present engaged in testing still further this question and we soon hope to be able to decide it definitely.

Lack of oxygen is, therefore, in all probability a very important factor in the death of the central tissues. There exist, however, certain other factors which have to be taken into account. Here also we shall at present restrict ourselves to a consideration of the kidney. The kidney is a conglomeration of different structures which possess different states of equilibrium. The straight tubules and the larger vessels seem to be more resistant than the majority of the convoluted tubules. We may see a straight tubule preserved when the surrounding convoluted tubules are dead. The glomeruli also, and especially their capsule is somewhat more resistant than the convoluted tubules, at least as far as their condition of preservation can be recognized on the basis of their structural features. As far as the life of these structures is concerned, their equilibrium is somewhat more stable than that of many convoluted tubules; they differ, however among each other in regard to growth phenomena. While mitoses are readily observed in pieces of kidney kept in culture media, in tubule and also in connective tissue cells, especially under an atmosphere of oxygen, only once was I able to observe a mitosis in a glomerulus and this occurred in its capsule. I have never been able to see mitotic division in blood vessels kept under these conditions, although blood vessels are relatively resistant. The states of equilibrium for growth on the one hand and life on the other hand, differ, therefore, in different structures. This holds good,

if we include into our consideration the occurrence of amitoses which can occasionally be found in tubule and probably also in connective tissue cells, under these experimental conditions.

The equilibrium of organs and tissues depends upon other internal factors which we are in a position to modify. We found that the regenerating kidney of the rabbit, into the surface of which five or eight days previously many cuts had been made is more resistant to injurious influences than the kidney in a resting state. The newly formed tubules seem to be especially resistant and in such a kidney we find more proliferation of connective tissue and of tubular epithelia than in a nonregenerating kidney. Whether this holds good also in the case of other organs will have to be tested in further experiments. Several years ago I found that regenerating epithelium of the skin did not give rise to more extensive growth after transplantation. Still it may be that regenerating skin also is more adjusted to changes of environment than normal skin. From the point of view of adaptation of living matter to its needs under normal and abnormal conditions it can be easily understood that tissues that have to adapt themselves to the unfavorable conditions found in and near a wound should be provided with a greater resistance to injurious influences such as we actually observed in the case of the kidney.

After the preceding analysis of some of the factors determining the life and growth of tissues in culture media, we have to inquire into the conditions that lead to movements of tissues. At the time I made my first experiments on the growth of tissues in culture media it was most generally believed that epithelial cells do not possess the faculty of locomotion but that their movements are due either to the spreading out of a formerly compressed elastic plate or to the passive pushing forward of cells as a result of mitotic division. I observed that epithelial cells are able to move into coagula—I used especially coagulated blood serum and fetal skin—in various directions, in rows of single cells as well as in cell columns with long drawn out cytoplasmic processes and concluded, therefore, that notwithstanding the mitotic division of epithelial cells in culture media which I discovered, these changes must be due to spontaneous movements of the cells. Further-

more, I noticed in these experiments as I had in previous observations, that the cells grow principally, if not altogether, in contact with solid bodies and on the basis of a few additional isolated observations which I found in the literature, mainly concerning the movements of connective tissue cells, I attributed to various kinds of cells a responsiveness to stereotropic stimulation. Since then we have extended our observations on the growth of tissues in culture media and we can state that we found in other tissues the same reactions which we had previously observed in the case of the fetal skin. We found that ordinary connective tissue cells, cells of myxoid connective tissue, cells of mouse carcinoma, and probably also the tubule cells of the kidney move in a similar way as the epithelial cells of the skin, namely in contact with the surface of the coagulum or in the coagulum and here also usually in the direction of and in contact with the fibers which quite commonly are produced in blood coagula under the influence of mechanical pull. The movement over the surface of the coagulum is carried out with greater rapidity than into the interior of the coagulum, the resistance to the advance being much greater within the coagulum.

There exist, however, great differences in the ease and readiness with which different tissues penetrate into the coagulum. Regenerating connective tissue perhaps penetrates most easily, then follows our mouse carcinoma which in its mode of movement resembles very much the movements observed by me formerly in the case of the epithelium of the skin. I also believe that tubule cells of the kidney grow into the coagulum; at least we have observed some pictures which we interpreted that way. However, they certainly move with greater difficulty than the other cells mentioned.

There are some possible errors in interpreting certain microscopic appearances. A partial retraction of the coagulum may take place which pulls with it the adherent layer of regenerated kidney tubule cells, covering the surface of the kidney. This layer then follows closely all invaginations of the coagula. But other appearances must in all probability be interpreted as actually due to an immigration of tubule cells, again in the direction

of the fibers of the coagulum. I have not, however, under ordinary conditions, observed the active penetration of regenerating kidney tubules as such into the coagulum. Sometimes appearances suggesting such an interpretation can be seen, but they are most probably due to an inclusion of preformed tubules into the fluid during the process of coagulation.

We have not observed a growth of the parenchymatous structures of the testicle or of the follicular tissue of the ovary into the coagulum, neither did I succeed in a single instance to notice the ingrowth of blood vessels into the coagulum, although they may remain alive. We know that new blood vessels grow with great readiness into wounds and in most places where active growth processes take place. Perhaps the absence of blood pressure in tissues growing in culture media is the factor that in this case is missing and without which the outgrowth of new vessels does not readily take place. In this connection I may mention that while new vessels grow very rapidly and abundantly into newly forming corpora lutea in the direct environment of which the blood vessels are well filled with blood, they grow only in exceptional cases into the interior of an atretic follicle, while the follicular cavity is being organized by ingrowing connective tissue cells. Accordingly we notice that the blood vessels around a follicle in the process of atresis are not well filled with blood.

By what process do the various cells penetrate into the coagulum? We can quite frequently observe the sending out of pseudopodia from the surrounding tissue directly into the coagulum in the case of connective tissue, tumor and also kidney tubular cells. In the case of tumor cells, I saw pseudopodia many times longer than the ordinary cell size. Within the coagulum also, we may occasionally notice that one single cell in contact with several fibers of the coagulum sends out several processes, each one in contact with a fibril. Cells may enter either as single rows or in solid columns into the coagulum. A row of tumor cells may in the coagulum branch off in various directions, different individual cells following different fibrils.

There exist also some differences in the locomotion of the different varieties of cells.

Connective tissue cells penetrate into the coagulum rather independently of each other, connected merely by long pseudopodia, or at other times apparently entirely disconnected. Carcinomatous cells or cells of squamous epithelium do not move as independently of their neighboring cells as connective tissue cells, they penetrate in columns or in single rows and may also occasionally round off or become otherwise independent of each other. Tubule cells of the kidney move usually in rows of connected low cuboidal cells, but may also apparently occasionally become somewhat more independent of each other. Ovarian follicles and the parenchyma of testicles do not move into the coagulum so far as we have been able to observe. On the basis of these observations we may conclude that there exists a close parallelism between the structure of tissues and their motility. Normally connective tissue cells are only loosely connected with each other; squamous epithelium and carcinoma cells are structurally more closely attached to each other. They form, however, plates free at both ends, while glandular organs form normally ring structures; still closer is the structural linking of cells in the case of the parenchyma of the ovary and testicle. Here we find rings of cells piled upon each other in several layers. The normal difference in the linking of the cells of various tissues to each other seems to be one of the factors determining their motility. The ease with which pseudopodia are sent out, seems in part, at least, to depend on the structural peculiarities of the tissues. Squamous epithelium of the skin and carcinomatous tissue of the mammary gland of the mouse resemble each other also in that respect that both may over a certain area penetrate diffusely into the coagulum, the individual cells breaking into the coagulum in various directions, surrounding the broken up pieces and taking them up into their cell body. The diffuse character of this breaking in into the coagulum being especially pronounced in the case of the tumor cells, but somewhat similar appearances are described also in my former work on the growth of epithelium in culture media.

Closely connected with the locomotion of the cells is the phagocytic power they manifest towards the coagulum. In a similar

manner I previously found that epithelial cells while penetrating into or lying in close approximation to the coagulum can take up solid particles of the coagulum into their cell body, first surrounding the piece with their cytoplasm; the same activity I found in the case of kidney tubule, connective tissue cells and the cells of the carcinoma of the mouse. Previously I described also phagocytic action of the sarcoma cells of the rat and dog, during ordinary processes of growth in the body. Active locomotion of the cells and phagocytic action depend probably on a similar mechanism.

What is the mechanism which enables the various kinds of cells to enter the coagulum?

If we compare the different ways in which cells penetrate into the coagulum and proceed mainly in the direction of its fibers, we may conclude that the cells act principally by mechanical and not by chemical means during these processes. They penetrate into the coagulum usually under certain conditions in which they find an opening into the coagulum. If the coagulum covers a layer of cells in such a manner that the long diameter of the cells and the direction of the fibers of the coagulum run parallel and the coagulum touches at the same time the flat surface of the cells, the cells do not usually penetrate into the coagulum. If on the other hand the direction of the fibers is at an angle with the flat surface of the cells, or if the pointed end of the cells is in contact with the coagulum in the direction of the fibers of the coagulum, the cells frequently migrate into it; especially do they enter crevices and rough surfaces of the coagulum, often in the form of a wedge, again turning in the direction of the fibers of the coagulum. The cells follow in the coagulum the path of least resistance and at the same time they remain as much as possible in contact with the fibrils. This could be especially well seen in cases in which we had added Ringer's solution to the fluid before it had coagulated. In such cases also a distinct moving of the cells in contact with the fibers takes place. Sometimes the cell columns cannot enter the coagulum, if the direction of the fibrils of the coagulum is unfavorable. In this case the cells may exert a pressure upon the coagulum, may bend it somewhat at a localized area without

penetrating through its surface layer. The cells evidently develop a certain kinetic force. By means of this kinetic force they penetrate into the coagulum. As a result of this pressure the coagulum is stained more reddish with eosin, wherever it is surrounded by various tissues. I do not wish to exclude the possibility of a secondary digestive action. Sometimes we notice that in the neighborhood of the moving cells the fibrillar character of the coagulum disappears, that the latter becomes homogeneous, staining more deeply red with eosin. Especially is this noticeable in pieces of coagulum surrounded by cellrows on several sides and particularly in pieces included in individual cells. It may be that these changes are not altogether due to pressure but that besides a digestive action takes place on the part of the cells.

As in the case of the life and multiplication of cells the extent of migration does not only depend on these external factors which we mentioned, but also on the state of the tissue. We found that the outgrowth which takes place from the regenerating is larger than from the resting kidney.

From all these observations we may conclude that it is especially the third class of phenomena namely the protoplasmic movements leading to migration of cells over wide areas and to some extent also to phagocytosis which presupposes the presence of and the contact with a solid or at least very viscous substance. In grouping these reactions of different cells together by designating them as stereotropic their mechanism has yet to be explained. I would like to offer an explanation of these phenomena which to a great extent is based on my former observations on the influence of environmental changes upon the movements and granules of the blood cells of Limulus. Here we find that contact with non-lipoid substances changes the constitution of the surface layer of the cells, it leads to stickiness of the formerly smooth surface layer and simultaneously to a sending out of pseudopodia. As a result of these changes, the cells adhere to and move in contact with the solid material.[6] The recent observations

[6] Virchow: Archiv, Bd. 173, 1903, p. 105. Biolog. Bull., vol. 4, 1903. Folia haemetologica, Bd. 4, 1907. Pflüger's Archiv, Bd. 131, 1910.

of Levaditi seem to show that also phagocytosis is preceded by changes in the surface layer of the acting cell which becomes more sticky and in consequence of this increased stickiness the particle to be included in the interior of the cells adheres in the beginning to the surface of the phagocyte. We may assume that also in this case the foreign particles acts upon the surface of the cell in a similar manner as the surface of glass upon the blood cell of Limulus. Both migration into the coagulum and phagocytosis seem therefore to depend upon essentially the same mechanism.

Just as we find in the case of the cells of Limilus, a certain ameboid activity in fluids that are not perfectly adequate, it is possible that a limited migration of certain cells may also take place without contact with solid bodies.

That this stereotropic mechanism is of great adaptive significance becomes clear when we consider the conditions of wound healing and inflammation, where an interesting interaction between coagulative phenomena and cell movements and phagocytosis is noticeable. The wandering of cells in contact with the coagulum is the foundation for the process of wound healing.[7]

We stated above that in cells while migrating in the coagulum mitoses can be observed. In this connection I made an observation which may not be without interest. We found that mitoses are never or if at all, certainly only extremely rarely found in connective tissue cells, which adapting themselves to the conditions in the coagulum, have become long drawn out and narrow, while mitoses in the same kind of cells which lie in a somewhat wider space in the coagulum are not altogether rare. Occasionally I got the impression that especially those cells that meet an obstacle during their migration over or in the coagulum and come to a standstill at such an obstacle, assuming here a rounded shape, are more liable to undergo mitotic division than other cells moving freely over the surface with long drawn out cytoplasm.

The various cells show no or only few anomalies during their migration in the coagulum. Occasionally we may find binucleated connective tissue, or also tubule cells of the kidney on

[7] Montreal Medical Journal, July, 1903.

or in the coagulum. Sometimes tubule cells at the edge of the kidney piece may enlarge, cytoplasm as well as nucleus. We might also refer to an apparent giant cell formation which we noticed in testicle put into coagulum,[8] furthermore to the fact that the parenchymatous structures of the testicle during their life in the coagulum do no longer produce spermatozoa. A similar anomaly I observed in my earlier work. The stratified epithelium of the skin loses the power to produce keratohyalim in the coagulum.

The method of growing tissues in culture media has recently been successfully extended to the study of invertebrate tissues. Mr. G. Harold Drew from the Marine Biological Laboratory in Plymouth, England, kindly gave me permission to refer to some of his experiments in which he cultivated certain epithelial tissues on agar. He noticed very interesting phenomena of cell-proliferation and phagocytosis, the epithelial cells behaving in a manner very similar to blood cells.

Further studies in this direction undoubtedly will yield results of great interest and importance.

The problems which can be attacked by the method of growing tissues in culture media are very manifold. It will I hope, ultimately lead to the building up of a physiology and pathology of tissues in a similar sense as we now possess a physiology and pathology of organs and I may add that this was the aim in my mind when I undertook my first experiments in this direction about fifteen years ago, and I may also add that to a great extent upon such a physiology and pathology of tissues the further analysis of morphological phenomena depends.

[8] Proceedings Soc. Exp. Biol. and Med., 8, 1911.

THE DEVELOPMENT OF THE BLASTODERM OF THE CHICK *IN VITRO*[1]

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TWELVE FIGURES[4]

Since Harrison's publication in 1907[5] in which he described the development *in vitro* of the nerve fibers of the frog embryo, experimental work in growing tissues has been largely confined to the culture of bits of tissue removed either from the embryo or adult animal or from various tumors. The development *in vitro* of the chick embryo, so far as we are aware, has not been described.

The rhythmical and vigorous contractions, for over five days, of the heart removed from a forty-hour chick embryo and planted in plasma, suggested to us the study of the developing vascular system. A seventy-two hour embryo was planted in plasma. The heart continued to beat uninterruptedly for over nine hours. During this time the entire vascular system could be seen in action under the microscope. While observing this first specimen we were impressed with the possibilities which such a method offers for the study of various problems in embryology and pathology. We began accordingly to test the viability and developmental possibilities of younger embryos. In this work we employed, in a modified form, the apparatus and technique which we have been using during the past year in growing adult and embryonic tissues.

[1] Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

[2] From the Department of Surgery, College of Physicians and Surgeons, Columbia University (The George Crocker Special Research Fund).

[3] From the Department of Surgery, College of Physicians and Surgeons, Columbia University.

[4] Expense of illustrations borne by authors.

[5] Anat. Record, vol. 1, 1907.

THE APPARATUS

The modified incubator is shown in the accompanying photograph (fig. 1). Its chief advantages are: First, observation of the specimen on a mechanical stage controlled from the outside, without alterations in temperature; Secondly, the opportunity offered to take low and high power photomicrographs of all or parts of the specimen with an accurately focused, condensed, and heat filtered light.

The incubator consists of two parts: a base below, above this the incubator proper containing the microscope. The base is a rectangular sheet-iron box similar to the usual type excepting for an air space which is obtained by means of a heavy iron plate placed 7 cm. below the under surface of the incubator. The purpose of this air space is to maintain a more equable temperature within the incubator.

The incubator proper, which rests upon an iron rim on the base, consists of a wooden box thoroughly insulated on its outer surfaces with asbestos. To its lower surface, is attached a perforated iron plate; the perforations are continuous with similar ones in the asbestos and wooden floor. These warm air inlets are plugged with glass tubes containing glass wool for filtering the air. Through the upper surface projects the draw tube of the microscope and immediately behind this is a small aperture for the extension rod connecting with the fine adjustment; to the right is a small door giving access to the incubator. A window in front transmits light to the mirror of the microscope. Above and to the side of this window is an opening for the controlling device of the Bean's heat regulator. At the back are two small openings, one above the other, for cords attached to the lever of the iris diaphragm. On the right side are extension rods controlling the coarse adjustment of the microscope and the two controls of the mechanical stage. On the left side is a door of ample size to allow free access to the incubator. Back of this passes an extension rod to control the substage. One arm of a right angled thermometer passes through this side and is so situated that its bulb lies immediately below stage of microscope. The tempera-

ture of the incubator is maintained by means of the small Bunsen burner in the base. The Bean's heat regulator as a thermostat has proved quite satisfactory as the temperature within the incubator seldom shows a variation of more than one degree.

The device here used for photomicrography consists, as is shown in the photograph, of a camera attached by means of a thumb screw to a slotted plate. This plate is kept rigid by being screwed to a frame work of iron piping, which in turn is securely fastened to the floor and braced. When taking photographs the camera is lowered to the lower limit of the slotted plate and the brass tube on the lens board of camera is inserted into the sleeve of the collar surrounding the draw tube of the microscope. When not in use the camera is disconnected from the microscope and pushed to the upper limit of the plate and clamped.

The method used for illuminating the field for photography is similar to that generally used in photomicrography, the only difference being that in this work the use of a heat filter is essential, for the concentrated heat rays from the small arc lamp, if not filtered, cause tissue death in a very few seconds.

THE TECHNIQUE

Fresh eggs are incubated at 37° to 39° C. Using aseptic precautions throughout, the blastoderm is removed from the egg by cutting wide of the area vitellina externa and lifting it out of the yolk with sufficient adherent yolk to prevent injury. The blastoderm is transferred to Locke's solution, kept at 37° C. on a water bath. Yolk granules and vitelline membrane are removed by gently squirting the solution against the blastoderm with a medicine dropper. The blastoderm is then floated onto a cover glass with its dorsal or upper surface in contact with the cover glass; the excess of Locke's solution is removed with sterile absorbent cotton. A few drops of plasma are placed on the blastoderm and when this has coagulated the cover glass is inverted over a hollow glass slide, containing a drop of water, and rimmed with paraffine. The specimen is then incubated at 38° C.

Plasma is obtained from the adult fowl by the method described by Carrel and Burrows, that is, blood is drawn from the external jugular vein through a glass canula, coated with olive oil, into ice cold paraffine tubes. The blood is centrifuged and the plasma is then refrigerated. We have used plasma either pure or mixed in different proportions with Locke's solution. When used unadulterated the specimen is securely fixed to the cover glass. This obviates the sagging and blurring of the specimen. The disadvantage in its use is the mechanical resistance which the jelly-like film, with its fibrin network, necessarily offers to the free expansion of the embryo in its growth. By means of special glass slides in which the blastoderm rests on a depressed disc in a mixture of Locke's solution and plasma or serum the hanging drop method is avoided. We hope to get better results by this means, which we have only recently employed.

The chief factors in preventing prolonged growth in our series of blastoderms have been:

1. Injury from rough handling, drying or cooling.
2. The limited supply of oxygen and nutriment. This difficulty we expect to overcome, in part at least, by improvements in the chamber.
3. The prolonged or too frequently repeated exposure of the blastoderm to the strong light in making photomicrographs. The plasma in many such cases liquefied much more rapidly than usual.

OBSERVATIONS

Our attempts to grow embryos before the appearance of the head fold have been unsuccessful, largely because of the difficulty of removing the blastoderm from the egg at such an early stage. But from the 3 – 4 somite stage up to 17 – 18 somites we have been able to watch continuous development. Embryos removed after the beginning of the heart beat, the 10 – 12 somite stage, have lived a variable length of time, the longest thirty-one hours. Comparing our specimens that have grown, as determined by the increase in somites and development of the nervous and vascular systems with those removed from the egg at corresponding stages

of incubation it is evident that development *in vitro* is practically the same as *in ovo*.

By this method continuous observations, hitherto impossible, can be made in the development of the primary divisions of the brain, the optic and the otic vesicles, the relation of the folds of the amnion to the splanchnopleure and somatopleure, the folding of the heart, with the cephalic progression of the auricles, and the process of somitic division. It is in the field of angiogenesis, however, that we believe the study of the blastoderm *in vitro* offers special opportunities.

We wish to make a preliminary report on observations made in the blastoderm before the establishment of well formed blood vessels.

In the area pellucida, with the embryo at the 3–6 somite stage, spaces appear, first at the margin of area opaca, shortly afterward in area pellucida and margin of embryonic body wall. The best place to observe them is in the area pellucida. Here they appear at first as isolated spaces, of various shapes and sizes, in an undifferentiated layer of mesenchymal cells beneath the ectoderm (fig. 7, *1*, *2*, *5*). These spaces are frequently bounded by a mere line, more or less refractile in character (fig. 7, *1* and 2). In others the lumen is lined with rounded or oval cells which later become fusiform and flattened (fig. 8, *2* and *4*). In not all of these spaces is there a well defined limiting wall. Many of them end blindly in undifferentiated mesenchyme (fig. 7, *1* and *5*).

Under observation, these isolated spaces have been seen to change their shape, to expand, and to unite with similar spaces (figs. 9, 10). In some specimens this union of isolated spaces is quite a rapid one and, unless observed during a limited period the process cannot be seen. After the 10 somite stage we have been unable to detect isolated spaces. With the confluence of spaces channels are formed (fig. 11). At times they show a fairly well defined lining of flattened cells. Under the strain of increasing fluid content these channels, situated as they are in the soft ooze of mesenchyme, dilate and a bulging of their walls occurs in their weaker parts. Where the bays, or recesses, of adjacent channels meet a communication is established and a plexus is

formed. This plexus formation is first noted at the level of the anlage of the omphalo-mesenteric veins (fig. 4, *2*). With the establishment of the heart beat plexus formation progresses rapidly. A remarkable example of the effect of the hydro-dynamics of the pumped fluid in these channels was seen in No. 36 of our series. After the heart had been beating steadily for five hours the plasma covering the blastoderm liquefied and the embryo sagged, while the area opaca remained adherent to the cover glass. This caused a kinking of the left omphalo-mesenteric vein. Immediately many of the channels in the area pellucida tributary to the kinked vein became engorged and the process of bulging of the walls in their weaker parts, with the formation of new channels became strikingly apparent. In two instances the walls of parallel channels were pushed together. In one case, after the walls had been in contact a half hour a new opening formed and blood cells rushed from the vessel of greater blockage into the more rapid stream of the other vessel. The same specimen showed vessels which had collapsed, owing to the shifting of the blood stream to other channels. Within an hour the cells lining these channels had lost their endothelial character and reverted to the undifferentiated type of mesenchymal cell.

The presence of a fluid in these channels was well demonstrated in several of our specimens. For prior to the establishment of the circulation isolated cells or groups of cells were seen changing their position in the lumen of the channels. With the onset of the heart beat there began a to and fro motion of these cells. Finally, as the circulation became established in a portion of the plexus these cells, or groups of cells breaking off from cell masses, suddenly shot through the channels as if an obstruction was suddenly removed. In the plexus, in the neighborhood of the omphalo-mesenteric veins, the direction of travel of these first cells was always from the area opaca to the sinus venosus. In one of our series (figs. 5 and 6) we were able to observe the development of the plexus in this region, the appearance of the heart beat and of the first two folds of the heart, the early oscillating movements of blood cells with their later streaming toward the

sinus venosus, and finally the passage of blood cells from the area opaca, through the plexus, to the sinus venosus, through the heart, down the left dorsal aorta, out of the vitelline artery, back to the area opaca.

The phenomena of the development of isolated spaces in the area pellucida; their confluence, resulting in channels; the elaboration of a plexus from these channels; the effect of the hydrodynamics of fluid, pumped by the heart through these plexuses, in establishing well defined blood vessels have been observed by us in our blastoderms. In some cases several of the stages have been watched in the same embryo. As yet we have been unable to follow all of the successive stages in the same specimen.

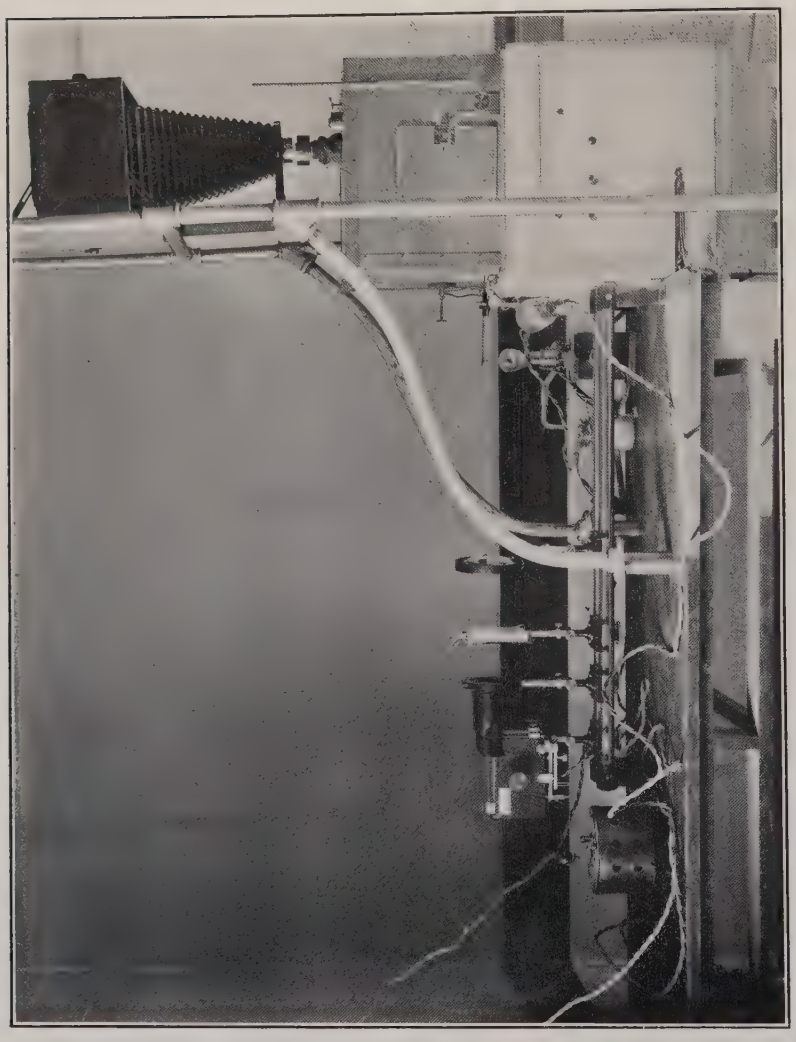

Fig. 1 Photograph of modified incubator.

Fig. 2 Photomicrograph of embryo at 6 – 7 somite stage. Note incomplete closure of neural fold. This specimen lived eighteen hours. × 28.

Fig. 3 Photomicrograph of same embryo as in fig. 2 shows 10 – 11 somites. Note development of fore brain. This specimen developed heart beat. × 28.

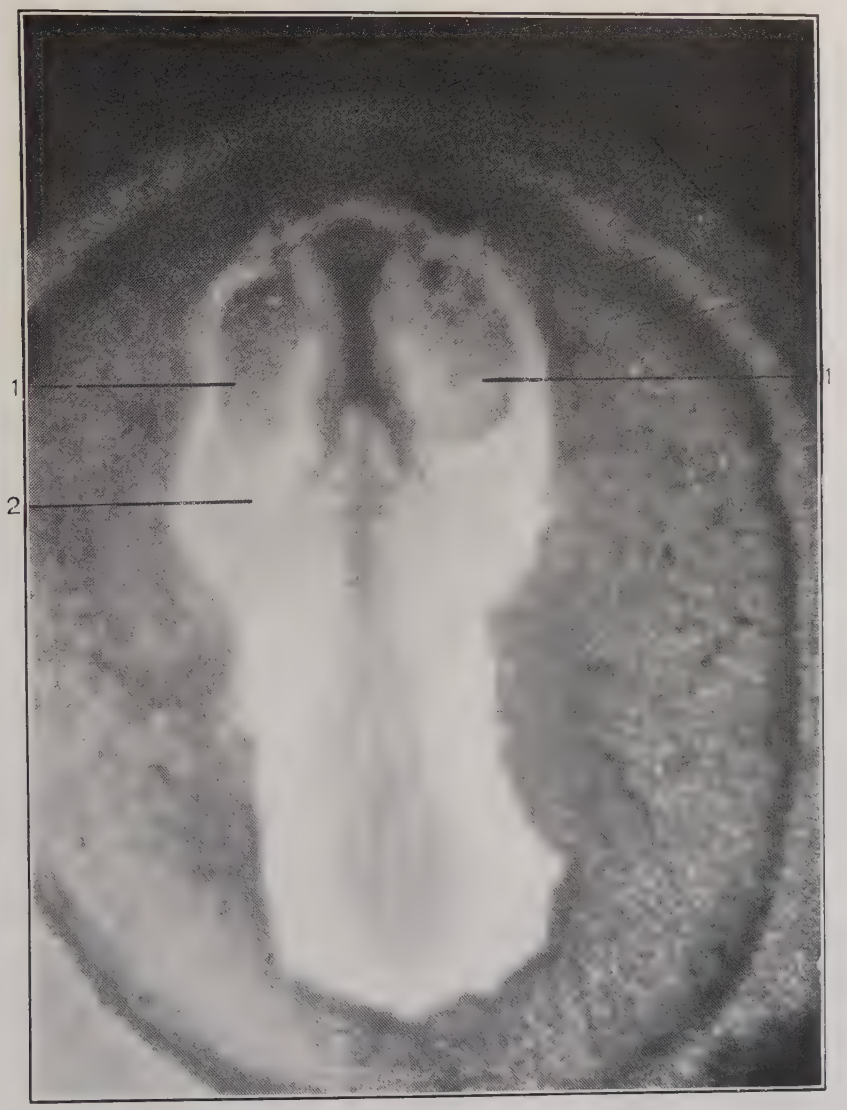

Fig. 4 Photomicrograph of embryo showing amnio-cardiac vesicles. × 28.
1 – 1.

Fig. 5 Photomicrograph of embryo showing 12 – 13 somites. Heart and partial circulation developed under observation. × 28.

Fig. 6 Photomicrograph of same embryo 14 – 15 somites with further folding of heart. × 28.

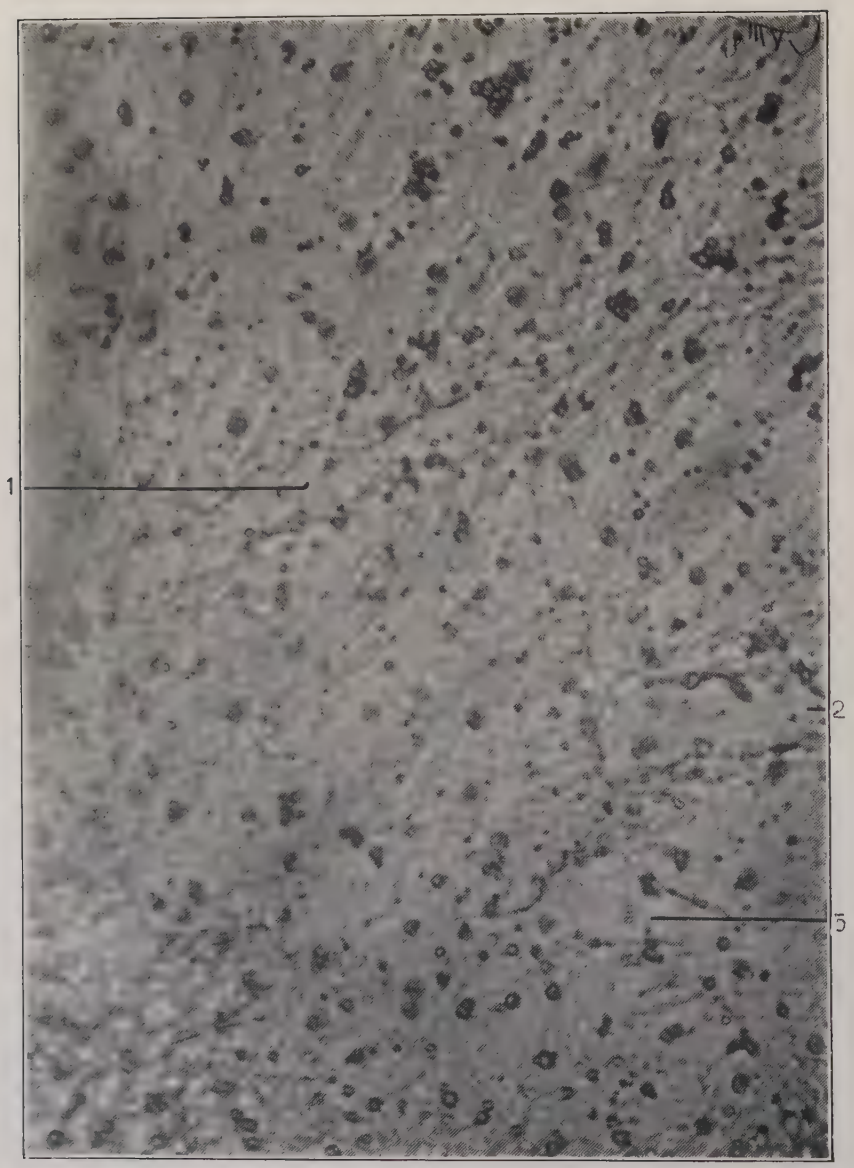

Fig. 7 Photomicrograph of isolated spaces *1 – 2 – 5*. These walls are refractile showing few cells. Note merging of space *5* in undifferentiated mesenchymal cells. × 500.

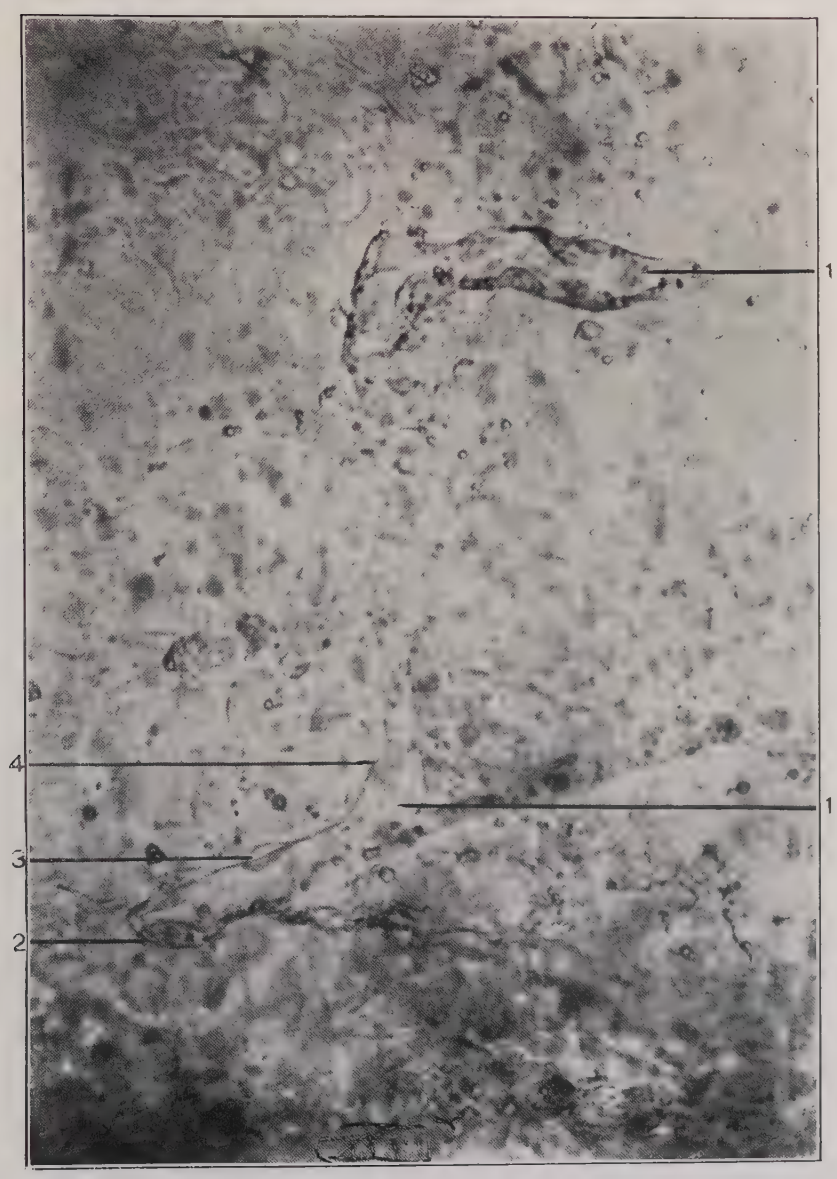

Fig. 8 Photomicrograph of two isolated spaces *1 – 1*. Note various shapes of cells *2 – 3 – 4* lining the spaces. × 500.

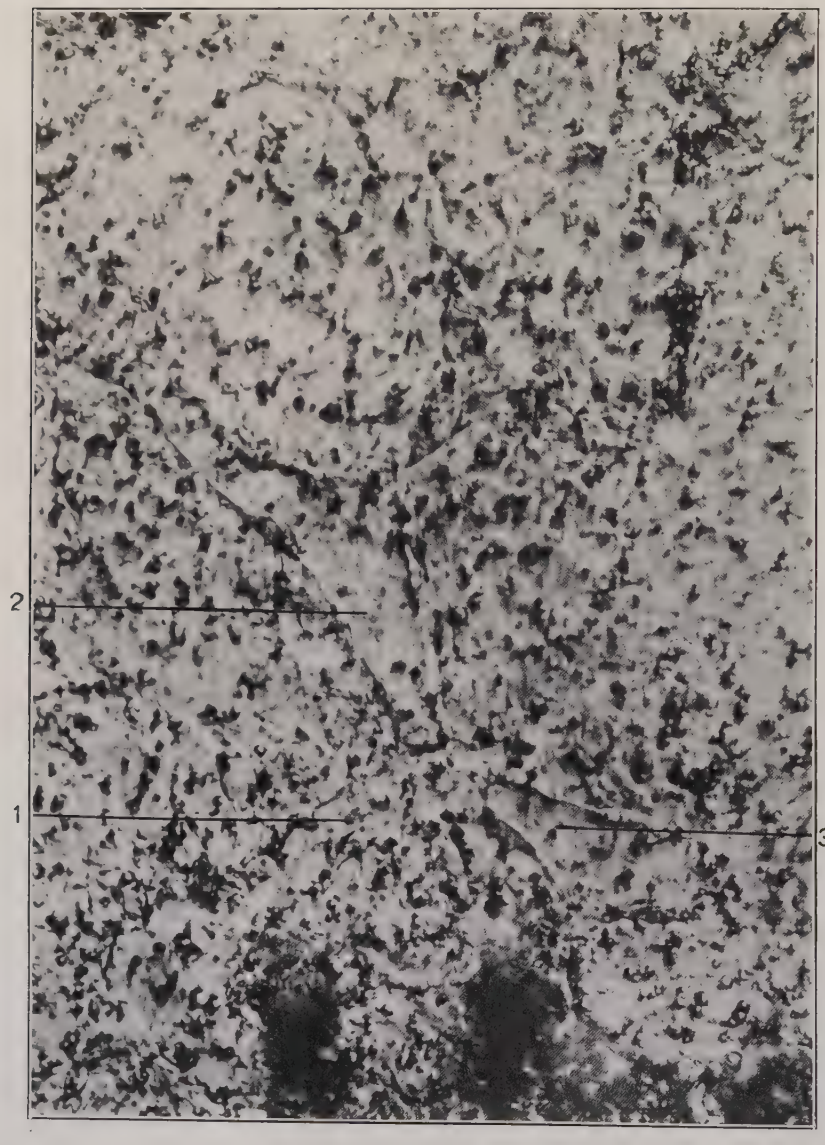

Fig. 9 Photomicrograph of isolated spaces *1* – *2* – *3*, about to unite. × 500.

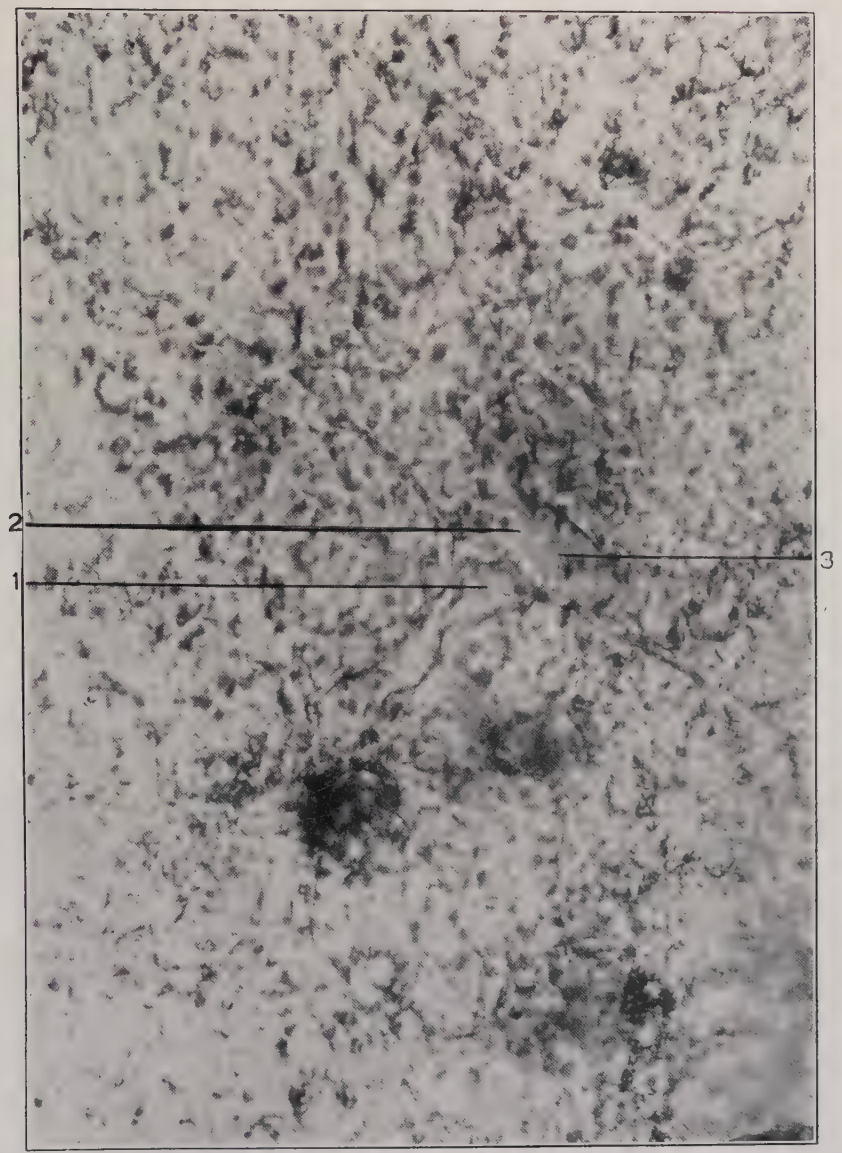

Fig. 10 Photomicrograph of same spaces *1 – 2 – 3*, as shown in fig. 9 now united. × 500.

Fig. 11 Photomicrograph of channels. × 500. 1.

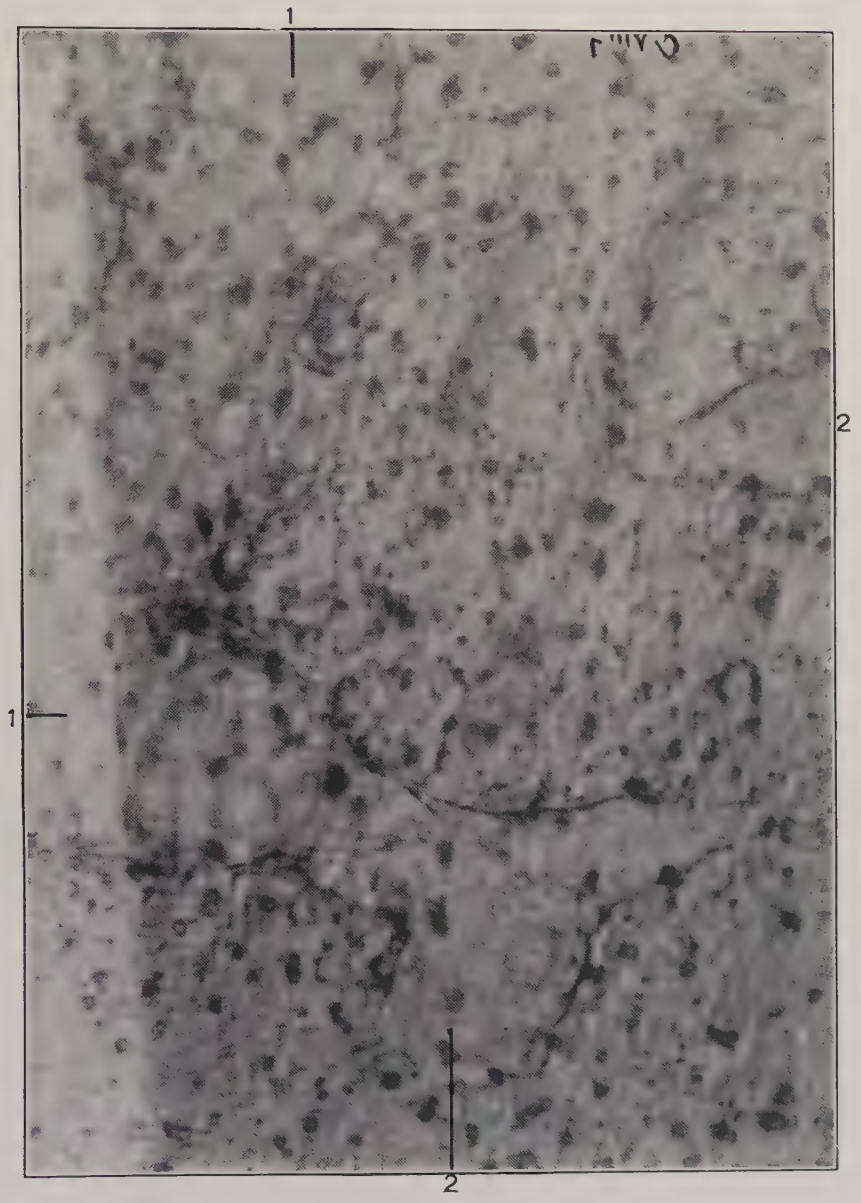

Fig. 12 Photomicrograph of channels *1 – 1* with evaginations *2 – 2* going on to plexus formation. × 500.

A METHOD OF FURNISHING A CONTINUOUS SUPPLY OF NEW MEDIUM TO A TISSUE CULTURE IN VITRO[1]

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ONE FIGURE

The method devised by Harrison for the cultivation of tissues in vitro has demonstrated that many varieties of tissues may survive and grow for a period of time outside the animal organism. Harrison originally devised this method for studying conditions surrounding the early differentiation of the nerve fiber and for this purpose it has answered admirably as it probably will for the differentiation of many other tissues. During the last year and a half this method with its modifications has been used by many workers as a means of studying the effects of salts and various animal extracts and fluids upon growth. The experiments of this kind have shown that absolute and constant differences, expressed in terms of rate and extent of growth are not obtained when identical physical conditions surround in each case the growing tissue unless the media used in these experiments are of widely different composition and contain substances which act promptly on the cell (cytotoxines, etc.). Such a result is to be expected in cultures where the period of great activity is immediate and endures for only a short period of time. The tissue vitality is undoubtedly sufficient to allow a considerable activity even in a medium which would ultimately bring about cellular death. Again, the chemical composition of the medium necessarily changes continuously throughout the period of cellular activity. In such cultures no sustained rate of growth is to be expected even if an ideal medium be employed. It was necessary, therefore, to improve the technic so that growth might

[1] Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

be continued over a longer period and render the growth more nearly comparable to cellular activities in the animal body. In this way problems of cellular metabolism and problems dealing with tissue growth and differentiation in various media may be attacked.

The first step toward such improvements has resulted in a method which supplies the tissue continuously with a known quantity of new media and at the same time removes the waste products without in any way disturbing the growing cells. The fresh medium is carried by means of a cotton wick from a reservoir at one end of a slide through a culture chamber and into a receiving reservoir situated at a lower level at the opposite end of the slide (fig. 1). In the culture chamber the wick is teased apart into its individual fibers which adhere to the surface of the cover glass and thus simulate a capillary system. The tissue is placed in this open network of cotton fibers and held there by a drop of coagulated plasma. The culture medium passes slowly along the wick through the culture and collects in the receiving chamber. The medium about the tissue is continuously changed by this means.

The supplying chamber (fig. 1, *a*) is blown from glass. It consists of two compartments, the horizontal or reservoir, and the vertical or wick chamber. These two chambers are connected by a glass tube, which comes from the bottom of the reservoir up and over to enter the upper end of the wick chamber through a small capillary point. The reservoir is open to the outside by a long vertical tube. The wick chamber is connected with the culture chamber (fig. 1, *b*) by two tubes. One tube carries the wick; the other acts as an air tube which equalizes the pressure on the two ends of the wick.

The rim of the culture chamber *b*, is made of cork. The central cavity of the cork is closed by a cover glass above and a long glass slide below. The tubes of the supplying chamber and receiving chamber enter through holes cut horizontally through this cork rim (see figure). All parts are carefully sealed together with paraffin. The wick coming from the supplying chamber is spread out on the under surface of the cover glass and here the culture is planted among the cotton fibrils.

The receiving chamber, fig. 1, *c*, is made of glass, and consists of a horizontal reservoir situated below the level of the slide. One tube connects this reservoir with the culture chamber and another tube opens to the exterior as shown in the figure. The wick passes from the cover glass through the glass tube to end above the surface of the liquid in the receiving chamber. The wick does not completely fill the tube. The arrangement allows free air communication between the receiving chamber and the culture chamber. The receiving chamber receives air by its communication

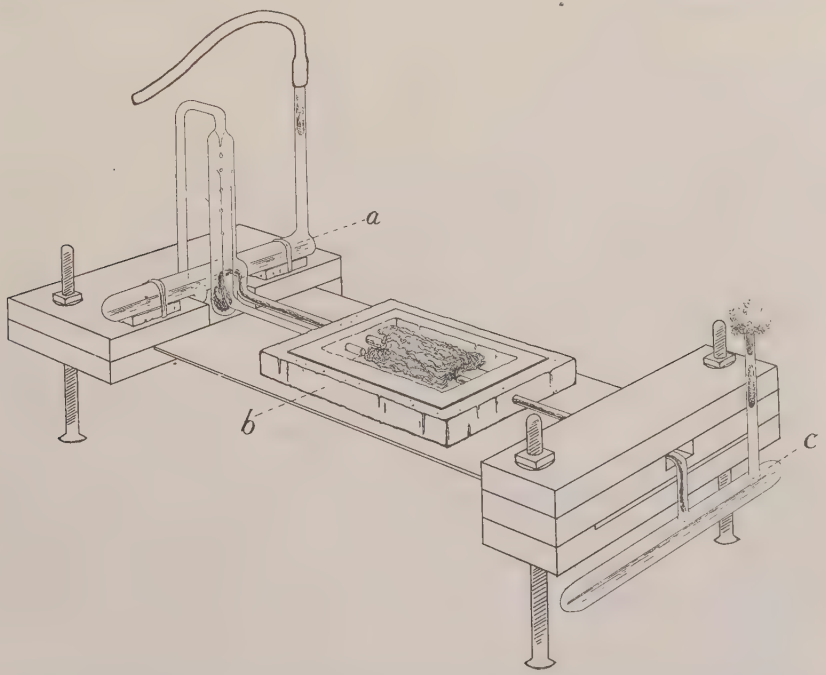

Fig. 1 Complete culture apparatus with rubber tube which connects with air pressure; *a*, supplying chamber; *b*, culture chamber; *c*, receiving chamber.

with the exterior through the vertical tube which is plugged with loose sterile cotton.

Blocks of wood held together with bolts are fastened by friction rigidly to the glass slide. A stand for the apparatus and firm supports for the glass chambers are thus formed. The entire apparatus is compact and strong and the contained culture may be examined under the microscope.

The glass chambers with the wick inserted are sterilized in the autoclave. The cork is sterilized in hot paraffin. The cover glass and the slide are sterilized by dry heat. To set up the apparatus the cork is removed from the hot paraffin and drained, the chamber tubes are inserted into it and the cover glass sealed over its surface. The wick is teased apart on the inner surface of the cover glass and the tissue planted. The apparatus is then immediately placed on the slide and all connections sealed with paraffin, and the chambers are now fastened to the wooden supports.

The reservoir of the supplying chamber is filled with the liquid medium and its open tube is plugged with dry sterile cotton. The open end of this vertical glass tube is connected with the pressure apparatus. The fluid is driven at a constant rate by air pressure from the reservoir into the wick chamber.

To refill or empty the chambers the vertical tubes are flammed, the cotton removed and the fluid entered or removed by sterile pipettes. Freshly sterilized cotton plugs are inserted and the apparatus again connected with the air pressure. Great care must always be taken to keep all parts of the apparatus and the media free from bacterial contamination.

Growth in such an apparatus has been tested with embryonic chick tissues in a medium of blood serum prepared from adult chickens. Growth of such tissues is vigorous and can be maintained for a considerable period of time. Hearts of embryo chicks and small pieces of heart muscle can be kept beating with great regularity. Small bits of ventricle beat actively until all muscle fibers have wandered apart and are lost in dense connective tissue outgrowths.

With this apparatus I have been able to test the effects of various media on the growing cells during any period of their activity. The growth is first established in control media. The media to be tested is then added and its effects recorded. In this manner the effects of many media may be tested on the growing cells without in any way disturbing or changing their physical surroundings. Changes in the chemical composition of the media can be tested by a comparison of the original media with samples obtained from the receiving chamber.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

TWENTY-EIGHTH SESSION

In the Palmer Physical Laboratory, Princeton University, Princeton, New Jersey, December 27, 28, 29, 1911

WEDNESDAY, DECEMBER 27, 10 A.M., TO 1:30 P.M.

The twenty-eighth session of the American Association of Anatomists was called to order by President George A. Piersol, who spoke of the loss this Association had met with, during the past year, in the death of Professor Thomas Dwight, for many years Parkman Professor of Anatomy, Harvard Medical School. In concluding his remarks the President appointed George S. Huntington to prepare a memorandum setting forth Professor Dwight's relations to the American Association of Anatomists and to Anatomical Science.

The following committees were appointed: *Committee on Nominations:* G. Carl Huber, *Chairman,* Frederic T. Lewis, Hermann von W. Schulte.

Auditing Committee: Robert J. Terry, *Chairman,* Jeremiah S. Ferguson.

The following papers were presented:

ROBERT BENNET BEAN, *Tulane University.* (a) The omphalic index. (b) Craniofacial indices. (c) Length relations of the body parts. (d) Morphology of the human pinna. Lantern. Read by title.

J. S. FERGUSON, *Cornell University Medical College, New York City.* The behavior and their relation to histogenesis of the living connective tissue cells of fish embryos.

THOMAS H. MORGAN AND C. PACKARD, *Columbia University.* The influence of radium on the development of eggs of Nereis, Arbacia and Ctenolabrus. Read by title.

SYMPOSIUM ON TISSUE CULTURE

Ross G. Harrison, *Yale University.* Cultivation of tissues in extraneous media as a method of morphogenetic study.

M. T. Burrows, *Cornell University Medical College, New York City.* Newer aspects of tissue culture *in vitro.*

John E. McWhorter and A. O. Whipple, *Columbia University, New York City.* The development of the blastoderm of the chick *in vitro.* With lantern demonstrations.

R. A. Lambert, *College of Physicians and Surgeons, New York City.* Morphological studies of tissues growing in culture media, with special reference to giant cells.

Margaret Reed Lewis, *Johns Hopkins University.* Membrane formation in artificial media. Presented by Warren H. Lewis.

Warren H. Lewis, *Johns Hopkins University.* The cultivations of tissues in media of known chemical constitution.

Leo Loeb, *Barnard Free Skin and Cancer Hospital, St. Louis.* A summary of observations on tissue growth *in vitro* and *in vivo.*

John Sundwall, *Johns Hopkins University.* Some observations on mammalian tissue growth. Read by title.

Alexis Carrel, *Rockefeller Institute.* New developments of the method of cultivation of tissues. Read by title.

2:30 to 5:30 p.m. Demonstrations in Guyot Hall. (See announcement of demonstrations.)

8:30 p.m. Smoker, at the Princeton Inn, in conjunction with the American Society of Zoölogists and the American Society of Naturalists.

Thursday, December 28, Palmer Physical Laboratory, 9:00 a.m. to 1:30 p.m. Session for the reading of papers, President George A. Piersol presiding.

Charles W. Green, *University of Missouri.* The disposal of fat in a new type of fat-storing muscle. Read by title.

A. J. Brown, *Columbia University, New York City.* The development of the pulmonary vein in the cat. Lantern.

Albert Kuntz, *Iowa State University.* The development of the adrenals in the turtle.

H. E. Jordan and A. K. Steele, *University of Virginia.* A comparative microscopic study of the intercalated discs of vertebrate heart muscle. Lantern.

George L. Streeter, *University of Michigan.* The surface anatomy of the forebrain of the opossum. Lantern.

George S. Huntington, *Columbia University, New York City.* Some biogenetic factors in the lymphatic development of amniotes. Lantern.

A. M. Miller, *Columbia University, New York City.* Histogenesis of the periaortal lymph spaces in the chick. Lantern.

Otto F. Kampmeier, *Princeton University.* The value of the injection method in the study of lymphatic development.

Frank A. Stromsten, *Iowa State University.* The development of the anterior lymph sacs in the loggerhead turtle. Read by title.

Alexander S. Begg, *Drake University, Des Moines, Iowa.* The anomalous persistence in embryos of portions of the peri-intestinal rings formed by the vitelline veins.

Walter Ranson, *Northwestern University Medical School.* The structure of the spinal ganglia. Lantern.

Henry H. Donaldson, *Wistar Institute of Anatomy.* A comparison of European and American Norway and Albino rats in respect to weight of the central nervous system.

John L. Bremer, *Harvard Medical School.* The early development of the aorta in the rabbit. Lantern.

Benjamin F. Kingsbury, *Cornell University.* (a) A note on the branchial organs in the human embryo. (b) The amphibian tonsils.

The following papers, read by title at the Wednesday morning Session, were, on recommendation by the executive committee, placed at the end of the announced Thursday morning program:

Robert Bennet Bean, *Tulane University.* (a) The omphalic index. (b) Craniofacial indices. (c) Length relations of the body parts. (d) Morphology of the human pinna. Lantern.

John Sundwall, *Johns Hopkins University.* Some observations on mammalian tissue growth.

Thursday, December 28, 2:30 p.m. Business Meeting, Palmer Physical Laboratory

The Secretary reported that the minutes of the twenty-seventh session were printed in full in the *Anatomical Record,* Vol. 5, pages 90 to 97, and asked whether the members present desired to have these minutes read. On motion the minutes of the twenty-seventh session were approved as printed.

The Treasurer made the following report for the year 1911:

Balance on hand December 23, 1910	$179.48	
Receipts for the year 1911	1375.20	
Total		$1554.68
Expenditures for the year 1911:		
Expenses of the Secretary-Treasurer, Ithaca meeting	$30.56	
Smoker, Ithaca meeting	18.69	
Postage	36.00	
Printing, typewriting, envelopes	19.95	
To 258 subscriptions to Vol. 11 of the *American Journal of Anatomy* and Vol. 5, *Anatomical Record*, @ $4.50	1161.00	
To 5 subscriptions to Vol. 12, *American Journal of Anatomy*, @ $5.00	25.00	
Total		$1291.20
		$263.48
Balance, deposited in the name of the American Association of Anatomists, at the Farmers and Mechanics Bank, Ann Arbor, Michigan, December 23, 1911		$263.48

Robert J. Terry reported for the Auditing Committee as follows: "We have examined the accounts of G. Carl Huber, Secretary-Treasurer, for the year 1911, showing a balance of $263.48 and found them correct." (Signed) Robert J. Terry, Jeremiah S. Ferguson.

On motion of Warren H. Lewis, the report of the Treasurer and of the Auditing Committee were accepted and adopted.

The Committee on Nominations through its chairman, G. Carl Huber, placed before the Association the following names:

For President, Ross G. Harrison, Yale University.

For Vice-President, Thomas G. Lee, University of Minnesota.

For members of the Executive Committee for the term expiring 1915, in place of Simon H. Gage and Warren H. Lewis, whose terms of office expired, Irving Hardesty, Tulane University and Henry McE. Knower, University of Cincinnati.

On motion the Secretary was instructed to cast a ballot for the election to the respective offices of the names presented by the Committee on Nominations. Carried.

The following were recommended by the Executive Committee for election to membership in this Association:

A. J. Brown, Demonstrator of Anatomy, *Columbia University, New York City.*

Martin Rist Chase, Assistant in Anatomy, *Northwestern University Medical School, Chicago.*

Hal Downey, Assistant Professor of Histology, *Department of Animal Biology, University of Minnesota.*

Otto Frederic Kampmeier, Graduate Student, *Princeton University.*

John E. McWhorter, Worker under the Crocker Research Fund, *Columbia University, New York City.*

Sutherland Simpson, Professor of Physiology, *Medical College, Cornell University, Ithaca, New York.*

George Milton Smith, Associate in Pathology, *Washington University, St. Louis.*

Henry Carroll Tracey, Instructor in Anatomy, *Northwestern University Medical School, Chicago.*

Allen O. Whipple, Instructor in Clinical Surgery, *Columbia University, New York City.*

On motion of Thomas G. Lee, the Secretary was instructed to cast a ballot for election to membership in the American Association of Anatomists of applicants recommended by the Executive Committee.

The Executive Committee, by unanimous vote, recommended the election of Professor Moritz Nussbaum of the University of Bonn, to Honorary Membership in the American Association of Anatomists.

On motion Professor Moritz Nussbaum was unanimously elected an Honorary Member of this Association.

James Playfair McMurrich presented a verbal report on the progress made by the International Commission for the revision of Myological Nomenclature and requested that his resignation from the Committee appointed by this Association to act on the Commission be accepted, on the ground that he felt too much out of harmony with the methods and results of the Commission to be of further assistance in its work. After some discussion it was moved and seconded that the Report be accepted as presented and that the Committee be discharged. Carried.

The following statement, prepared by George S. Huntington, was read:

The Association of American Anatomists at its twenty-eighth session, held in Princeton, New Jersey, December 27, 28 and 29, 1911, desire to record the loss which American Science has sustained through the death of Professor Thomas Dwight of Harvard University. Professor Dwight, one of the founders of the American Association of Anatomists and one of its first presidents, devoted much time and earnest effort to the organization of this association. A very large part of its successful development is primarily due to these efforts and to the influence which Professor Dwight's sterling personality and sound scientific work exerted. His associates, while mourning the loss which American Anatomy has sustained, feel that the example and influence of his life and work remain to them as a force which will continue to aid scientific progress in this country.

On motion of Edw. Anthony Spitzka, seconded by Henry H. Donaldson, the Secretary was instructed to spread this statement on the minutes of this Association and send a copy of the same to Mrs. Dwight.

On motion the business meeting was adjourned.

3:30 to 5:30 p.m. Demonstrations in Guyot Hall. (See announcement of demonstrations.)

FRIDAY, DECEMBER 29, PALMER PHYSICAL LABORATORY, 9:00 A.M. TO 1:30 P.M., SESSION FOR THE READING OF PAPERS, PRESIDENT PIERSOL PRESIDING.

S. HATAI, *Wistar Institute of Anatomy.* Influence of exercise on the weight of the central nervous system and viscera of albino rats. Lantern.

J. PARSONS SCHAEFFER, *Yale University.* On the genesis and development of the nasolacrimal passages in man. Lantern.

ELIZABETH H. DUNN, *University of Chicago.* Reduced caliber in old age among the peripheral medullated nerve fibers.

VICTOR E. EMMEL, *Washington University Medical School, St. Louis.* The macrophages and other free cells in the serous cavities of the embryo.

H. VON W. SCHULTE, *Columbia University, New York City.* The development of the sublingual glands in the pig. Lantern.

FREDERICK TILNEY, *Columbia University, New York City.* The infundibular relations of the hypophysis cerebri. Lantern.

F. L. LANDACRE, *Ohio State University.* The epibranchial ganglia of Lepidosteus osseus. Lantern.

LEONARD W. WILLIAMS, *Harvard Medical School.* Notes on the osteology of the guinea pig.

HUGH D. REED, *Cornell University.* Some features in the development of the primordial cranium of the European hedgehog. Read by title.

CHARLES H. DANFORTH, *Washington University Medical School, St. Louis.* The heart and arteries of polyodon. (Presented by R. J. Terry.)

ULRICH DAHLGREN, *Princeton University.* Anal glands of mammals.

H. E. JORDAN AND J. C. FLIPPIN, *University of Virginia.* Haematopoiesis in chelonia. Lantern.

W. H. F. ADDISON, *University of Pennsylvania.* Observations on the development of the cerebellum of Bufo.

EDW. ANTHONY SPITZKA, *Jefferson Medical College.* The Daniel Baugh Institute of Anatomy. Lantern.

At the end of this session, with which the twenty-eighth session of this Association was brought to a close, Professor Charles F. W. McClure and the authorities of Princeton University, on motion, were tendered a vote of thanks for the very efficient arrangements made and for their hearty coöperation in furthering the success of this meeting.

DEMONSTRATIONS PRESENTED

Demonstrations of tissue cultures and of apparatus used in the same were made by Ross G. Harrison, Montrose T. Burrows, R. A. Lambert, Warren H. Lewis, John E. McWhorter, Allen O. Whipple.

ROBERT BENNET BEAN, *Tulane University of Louisiana.* Filipino and negro ears.

JOHN L. BREMER, *Harvard Medical School.* Drawings, slides and models illustrating the early development of the aorta in the rabbit.

MRS. E. R. CLARK, *Johns Hopkins University.* Injections of the posterior lymph hearts and allantoic lymphatics in chick embryos.

H. E. JORDAN AND K. B. STEELE, *University of Virginia.* Microscopic preparations illustrating the intercalated discs of vertebrate heart muscle.

H. E. JORDAN AND J. C. FLIPPIN, *University of Virginia.* Microscopic preparations illustrating haematopoiesis in chelonia.

BENJAMIN F. KINGSBURY, *Cornell University.* Models of the human pharynx.

ALBERT KUNTZ, *Iowa State University.* Microscopic preparations illustrating the development of the adrenals in the turtle.

F. L. LANDACRE, *Ohio State University.* Microscopic preparations illustrating the epibranchial ganglia of Lepidosteus osseus.

FREDERIC T. LEWIS, *Harvard Medical School.* Models of the embryonic pancreas.

FLORENCE R. SABIN, *Johns Hopkins University.* Silvered embryonic blood vessels in cleared specimens. (Presented by E. R. Clark.)

HAROLD D. SENIOR, *University and Bellevue Hospital Medical College.* Racks for transporting 2 and 3 dozen slides at a time, for expeditious handling of class paraffine material.

George L. Streeter, *University of Michigan.* (a) Models and sections showing surface areas of the forebrain of the opossum. (b) Drawings illustrating the general form and finer structure of the opossum brain. (c) Series of student drawings made in the course in Regional Anatomy as given at the University of Michigan.

Robert J. Terry, *Washington University Medical School, St. Louis.* Cross section anatomy.

John Warren, *Harvard Medical School.* Models of the paraphysal and pineal regions in sheep and man.

Leonard W. Williams, *Harvard Medical School.* Illustrations of the bones of the guinea pig.

G. Carl Huber,
Secretary-Treasurer.

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BIFID APEX OF THE HUMAN HEART

FRANKLIN P. MALL

From the Anatomical Laboratory of the Johns Hopkins University

THIRTEEN FIGURES

Several years ago a heart was found in our dissecting room with a cleft in its apex fully 2 cm. deep separating the two horns of the vortex. It seemed to me, at the time, that there must be an embryological explanation for this, which would also throw light upon the development of the fiber bundles of the left ventricle. In writing upon the musculature of the ventricle of the adult heart,[1] I advanced an hypothesis to explain the peculiar arrangement of the heart muscle fibers, especially those of the left ventricle. In a forthcoming paper[2] upon the development of the human heart it is my purpose to show that this hypothesis is correct, for in their development the first circular fibers of the left ventricle surround its venous ostium; these subsequently fall over one another, like the hoops upon a broken barrel, to form the muscle mass which I have termed the 'bulbo-spiral band.' In so doing these bundles become interlocked with a primary band which connects the two ventricles on the front side of the heart (the sino-spiral band), and these form the two horns of the vortex.

In the specimen with cleft apex there is no true vortex as the fig. 1 shows. The sino-spiral band, *S S*, crosses the posterior longitudinal sulcus, encircles the tip of the right ventricle and is lost there; high up on the anterior side of the heart fibers penetrate the depth at the bottom of the anterior longitudinal sulcus and spread out on the inside of the left ventricle. The bulbo-spiral

[1] Mall, Amer. Jour. Anat., vol. 11, 1911, Schema A.
[2] Mall, Ibid., vol. 13, 1912.

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band, *B S*, encircles the tip of the left ventricle and then passes up into the septum. The bands which are crowded together in the normal heart to form the vortex are here separated, that is, there is an arrest of development of the apex. A glance at the figures of the normal heart, especially Schema B, given in the previous paper will make this point clear. Had the apex of the right ventricle become adherent to the left early in development, the sino-spiral muscle fibers would have gained possession of the whole anterior surface of the left ventricle, and in their downward shift, the bulbo-spiral would have become interlocked with them to form the posterior horn of the vortex. In this specimen the

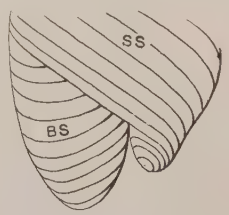

Fig. 1 Sketch of the lower part of an adult heart with a cleft apex showing the course of the bulbo-spiral, *B S*, and sino-spiral, *S S*, bands. One-fourth natural size.

bulbo-spiral occupies the entire tip of the left ventricle while in the normal it covers only the posterior side of the apex.

A slight cleft of the apex is not rare, for it is produced whenever the anterior and posterior longitudinal sulci meet; and Orth[3] states that a deeper cleft is of quite common occurrence. A similar statement is made by Ried:[4] "The two grooves (anterior and posterior longitudinal sulci) are connected with each other at or near the apex by a small notch, which is sometimes of sufficient depth to give the heart a bifid appearance."

The division of the ventricle is very marked in several marine mammals, thus in the dugong the cleft reaches nearly to the base, the two ventricles not being completely separated from each

[3] Orth, Path. Anat., vol. 1, 1887, p. 144.
[4] Ried, Todd's Cyclopaedia, vol. 2, 1839, p. 578.

other.[5] From time to time one reads the statement that the bifid form is embryonic, that is, that the embryo has a divided apex. In the literature the adult bifid heart is occasionally pictured,[6] or in rare instances the apex is formed by the right ventricle.[7]

I had an opportunity to test this question in the human embryo while studying the development of the heart. I studied about one hundred hearts in serial sections and twenty entire hearts which had been removed from the embryo to be dissected under the binocular microscope. In embryos up to 10 mm. in length the heart is still of amphibian form but shortly after this it takes

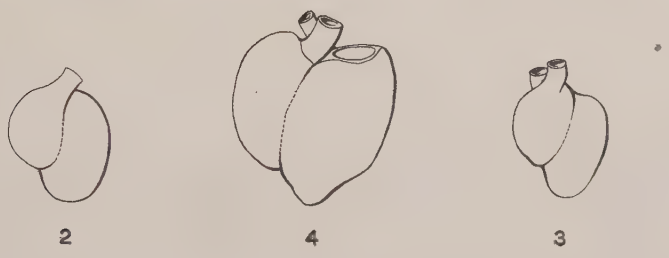

Fig. 2 Outline of the anterior side of a normal heart from an embryo 14 mm. long (No. 360).

Fig. 3 Normal heart, anterior view, from an embryo 18 mm. long (No. 90 b).

Fig. 4 Anterior view of a normal heart from an embryo 25 mm. long.

on the adult mammalian form. The transformation is practically complete at the time the interventricular foramen closes. At this time the interventricular groove is less pronounced, the hour-glass form is lost and the right ventricle does not reach the apex, that is, it is shorter than the left (figs. 2, 3, 4, 6, 7). However, in numerous specimens the adult form is not reached immediately and in them we may speak of a bifid apex. Of the large number studied there were eight specimens over 11 mm. long with divided apex. These embryos range in length from 11

[5] Todd's Cyclopaedia of Anatomy, vol. 1, 1835, p. 576; Owen's Comparative Anatomy, vol. 3, 1868, p. 521.

[6] For instance, Rokitansky, Die Defecta d. Scheidewand des Herzens, Wien, 1875.

[7] Merkel, Virchow's Archiv, vol. 48.

to 25 mm., showing that there is a tendency for the divided apex to remain for some time after the adult form should be present. The normal adult form was first present in a specimen 14 mm. long (figs. 2 and 6). Fig. 9 is a reconstruction of a bifid apex in an embryo 11 mm. long, and fig. 12 is from a coronal section of a divided apex in an embryo 24 mm. long.

When small hearts are dissected with the aid of the binocular microscope it is found that the two primary muscle bundles can be outlined in specimens 10 mm. long. At this time it is found that fibers arise from the sinus, cross the bulbo-ventricular groove behind, encircle the bulb and enter the left ventricle through the

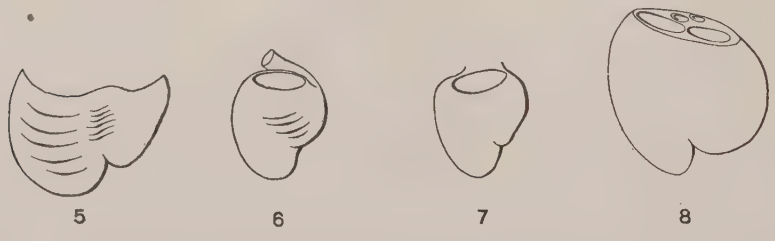

Fig. 5 Lower part of a heart from an embryo 12 mm. long. There is a slight cleft in the apex. The general course of the fibers on the posterior side is shown.

Fig. 6 Posterior view of the heart from an embryo 14 mm. long (No. 360). Normal.

Fig. 7 Posterior view of the heart, which is normal in form, from an embryo 18 mm. long (No. 90 b).

Fig. 8 Posterior view of a heart with a cleft apex from an embryo 19 mm. long (No. 283 b).

bulbo-ventricular groove in front. This is the sino-spiral muscle sheet. Another sheet arises from the sinus in front near the bulb, encircles the left ventricle and enters the septum behind (fig. 11). This is the bulbo-spiral band which may be outlined as soon as the inferior septum of the ventrical begins to develop from below and behind. In the dissection of somewhat older hearts the sino-spiral band is the easiest to isolate, and in its passage into the left ventricle it divides into two strands, one of which passes to the membranous septum on the right side of the septum and the other is lost in the trabecular system of the left ventricle. Between these two strands the circular strands (bulbo-spiral) of

the left ventricle may be seen. All this matches the arrangement in the mature heart as well as in the adult specimen with a bifid apex.

I have found a cleft apex in a young human embryo, apparently normal, that reminds me much of the adult heart in the dugong. The embryo (No. 434) was 15 mm. long, imbedded in an ovum measuring 30 x 28 x 20 mm. which was covered with beautiful regular villi. Upon careful inspection of the embryo it was found that the pericardial cavity was unusually large and within it the small and peculiar heart could be seen. The heart was removed, stained, and examined with the binocular microscope.

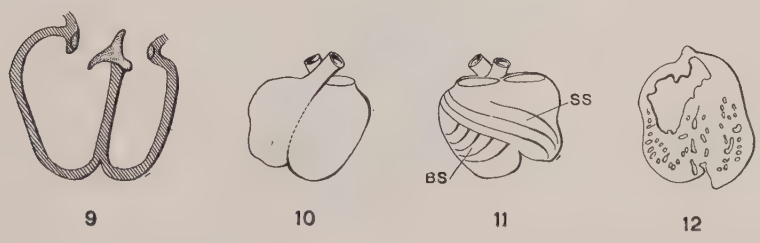

Fig. 9 Reconstruction of the walls of the heart of an embryo 11 mm. long (No. 353). The atrio-ventricular septum and the lateral endocardial cushions are dotted. The interventricular foramen is not shown. Enlarged 12 diameters.

Fig. 10 Anterior veiw of a bifid heart from an embryo 25 mm. long (No. 118).

Fig. 11 Posterior view of the heart shown in fig. 10. By dissection under the binocular microscope the course of the bulbo-spiral, *B S*, and sino-spiral, *S S*, bands of muscle fibers was determined.

Fig. 12 Coronal section of a bifid heart in an embryo 24 mm. long (No. 455). Enlarged 8 diameters.

The cleft in the septum is about complete, reaching nearly to the base (fig. 13). Apparently no fibers pass from one ventricle to the other and each is covered with its own circular fibers. In Canada balsam the cavities of the ventricles can be seen as they are partly filled with blood. It also appears as if an interventricular foramen were still present.

Therefore, it is proper to state that the bifid apex occurs as a frequent variation in small hearts and that in the course of development the anomaly is obliterated by the immense development downward of the two ventricles. It is probably more cor-

rect to speak of the downward development of the apex than the upward development of the interventricular septum, for this septum appears very early, while the heart is still in the amphibian stage, as a slight ridge of tissue within the heart opposite the interventricular groove. This also marks the position of the interventricular foramen. As the heart grows larger and larger for a time the foramen also becomes larger while the apices grow downward with a cleft between them. This is also the opinion

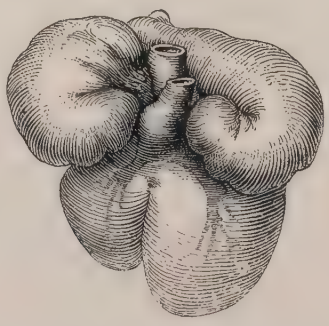

Fig. 13 Bifid heart from an embryo 15 mm. long (No. 434) anterior view. The outline of the ventricular cavity is shown. Enlarged 13 times.

of Flack.[8] Lastly the interventricular foramen is closed off by a slight growth upward of the inferior septum to form the membranous septum. The cleft apex then soon disappears, or remains as a slight notch between the ventricles. In rare instances it becomes very pronounced, as in this specimen, and in such cases there is no true vortex, as the sino-spiral band does not reach to the apex of the left ventricle but instead the bulbo-spiral band occupies the whole of the apex as shown in fig. 1.

[8] Flack, Further Advances in Physiology, edited by Hill. Longmans, New York, 1909.

SOME USEFUL MORPHOLOGIC FACTORS IN RACIAL ANATOMY

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After measuring several thousands of individuals, and making careful inspection of many thousands more, some Americans (white), some Europeans, some Filipinos, Negroes, Negritos, Chinese, Japanese, and East Indians, I have selected those factors that seemed to me to be most distinctive in relation to the development of the individual and the evolution of the race. A few of the most important of these factors will be presented below.

A. THE OMPHALIC INDEX

The position of the umbilicus varies from infancy to maturity, it varies sexually, it varies in different races and in different types in the same race. A factor that expresses this variation in terms of a single number is desirable, therefore I have devised what I call the omphalic index, which is obtained as follows: The distance of the umbilicus from the symphysis pubis is divided by the distance of the umbilicus from the suprasternal notch. This gives the height of the umbilicus from the pubis in terms of the distance of the umbilicus from the suprasternal notch, the latter always being 100. If the umbilicus is high, or far from the pubis, then the omphalic index will be high; and if the umbilicus be low, or near the pubis, then the omphalic index will be low.

A few general averages will suffice to illustrate the usefulness of the omphalic index as a factor in racial anatomy:

GROUP	NUMBER MEASURED	OMPHALIC INDEX
Igorot boys below the age of ten years	5	44.3
Igorot boys between the ages of ten and seventeen years	35	40.2
Igorot women	10	50.0
Igorot men	104	41.1
Taytay women	63	48.2
Taytay men	180	42.4
California women (Miss Johnson)	350	43.9
California men (Miss Johnson)	650	41.4

In any group of individuals the omphalic index varies according to the apparent stage of development of the individual. We may therefore segregate the individuals of any group into three classes, Hypo-onto-morph, or the less developed, Hyper-onto-morph, or the well developed, and the Meso-onto-morph, or intermediate. A few illustrations will suffice:

Women of Taytay (Filipinos)

TYPE	OMPHALIC INDEX
Hypo-onto-morph	48.4
Meso-onto-morph	47.6
Hyper-onto-morph	46.4

Men of Taytay (Filipinos)

Hypo-onto-morph	44.1
Meso-onto-morph	42.9
Hyper-onto-morph	41.4

Tulane students (white male)

Hypo-onto-morph	36.2
Meso-onto-morph	34.9
Hyper-onto-morph	34.1

Different groups of people represent different stages of development therefore the terms, Hypo-phylo-morph, etc., may be used. Examples:

GROUP	NUMBER MEASURED	TYPE	OMPHALIC INDEX
Women of Taytay.....	63	Hypo-phylo-morph	48.2
Women of Siberia..... (Jockelyn-Brodsky)	63	Meso-phylo-morph	42.0
Russian Jewesses (Teumin)..		Hyper-phylo-morph	33.0
Men of Taytay—Filipinos....	180	Hypo-phylo-morph	42.4
Men of Cainta—East Indians.	38	Meso-phylo-morph	39.6
Tulane students.....	55	Hyper-phylo-morph	35.1

As may be inferred from the preceding, the women of the groups presented may be called Hypo-morphs and the men Hyper-morphs, in relation to the position of the umbilicus.

B. CRANIO-FACIAL INDICES

The size of the face varies with age, sex, race and with inter-racial types. The size of the head varies so little that it may be used to compare with the size of the face, obtaining in this way an index that gives at once the relative and absolute size of the face.

The most complete index would be obtained by dividing the length plus the breadth plus the depth of the face by the length plus the breadth plus the height of the head, but all of these dimensions are seldom secured, therefore I have utilized the two dimensions of the face and of the head that are usually taken. The cranio-facial index that I have used is therefore the quotient of the chin to the nasion diameter plus the bizygomatic diameter of the face, divided by the glabella to the maximum occipital diameter plus the maximum transverse diameter of the head. It represents the size of the face in terms of the head size as 100. If the face is large the cranio-facial index is large and vice versa.

For example, among 1530 children in the public schools of Ann Arbor, Michigan, the cranio-facial index varied from 63.7 at five years of age to 72.2 at seventeen years of age for girls, and from 62.3 at five years of age to 73.5 at seventeen years of age for boys. Among 780 children in the public schools of Manila, P. I., the index varied from 66.8 at nine years to 73.8 at seventeen

years for girls, and from 63.8 at eight years to 74.2 at seventeen years for boys. Among 44 Igorot boys the index varied from 64.8 to 68.8 at nine and fifteen years respectively.

An interesting feature of the growth of the face as represented by the cranio-facial index is that the girls' face is larger than the boys' at an early age, after which it is passed by that of the boys; later it passes that of the boys but is again passed by that of the boys.

The face of the Filipino children, both sexes, has the same relation to the face of the American children that the American girl's face has to that of the American boy. The face of the Igorot boys is uniformly smaller than that of the other groups mentioned above.

Women have smaller faces than men as exemplified in the students eighteen to twenty years of age (female index 74, male 75), and in the women and men of Taytay, 74.3 and 75 respectively.

The cranio-facial index varies with race as follows:

GROUP	NUMBER MEASURED	TYPE	CRANIO-FACIAL INDEX
Men of Taytay	183	Hypo-phylo-morph	75.0
Men of Cainta	38	Meso-phlyo-morph?	75.7
Negro men	74	Hyper-phylo-morph	78.8

The types in each race vary according to development:

Tulane students

TYPE	CRANIO-FACIAL INDEX
Hypo-onto-morph	73.8
Meso-onto-morph	74.2
Hyper-onto-morph	76.1

American negroes

Hypo-onto-morph	75.1
Meso-onto-morph	78.8
Hyper-onto-morph	80.0

From the preceding it may be inferred that the negroes are Hyper-phylo-morph and the whites are Hypo- or Meso-phylo-morph in relation to the size of the face.

C. RELATIVE ARM AND LEG LENGTH

The total arm or leg length divided each by the stature gives the lengths of each of the extremities in terms of the stature as 100. These factors are known to increase with age, to be larger in men than in women and to be different in different races. They may be utilized to determine the position in phylogeny of a given people, and the position in ontogeny of a type within a group. For example:

GROUP	NUMBER MEASURED	TYPE	INDEX
Igorot men	104	Hypo-phylo-morph	Arm 44.0 Leg 51.6
Taytay men	180	Meso-phylo-morph	Arm 45.8 Leg 52.1
Negro men		Hyper-phylo-morph	Arm 46.6 Leg 53.3

Igorot men

TYPE	LEG INDEX	ARM INDEX
Hypo-onto-morph	51.5	43.8
Meso-onto-morph	51.6	43.9
Hyper-onto-morph	51.7	45.2

Taytay men

Hypo-onto-morph	50.8	45.6
Meso-onto-morph	51.7	46.3
Hyper-onto-morph	52.4	46.4?

Negro men

Hypo-onto-morph	51.5	
Meso-onto-morph	52.8	44.8
Hyper-onto-morph	55.1	46.2

The negroes represent advanced evolution in arm and leg, the Igorots, retarded. In each group there are individuals that represent advanced and retarded development.

D. THE HUMAN PINNA

Three types of the pinna have been found in every group of mankind that I have studied. The three types differ mainly in the helix and ant-helix systems around the lower part of the concha. One type is characterized by the inrolled helix and the depressed ant-helix which gives a trumpet like shape to the pinna; another type is characterized by the rolling out of the helix and the eversion of the ant-helix (tragus and antitragus); and the third type appears to be somewhat intermediate between the other two.

If the development of the pinna after the fourth month of fetal life consists of the rolling in of the helix followed by the eversion of the ant-helix, as suggested by Schwalbe, then the types of the pinna that I have discovered represent stages in the development of the human external ear.

Negroes, Europeans, Filipinos, Chinese, Japanese, East Indians and Negritos exhibit these three distinct forms of the human pinna: (1) *Hypo-onto-morph:* Depressed anthelix, inrolled helix, deep bowl or trumpet shape; (2) *Meso-onto-morph:* Intermediate; (3) *Hyper-onto-morph:* Everted anthelix, everted tragus and anti-tragus, rolled out helix.

SUMMARY OF FACTORS

The ear form or the shape of the pinna is not the only factor that makes these three types distinct. Enumerated below will be found other factors that stamp these types as entities:

HYPO-ONTO-MORPH	MESO-ONTO-MORPH	HYPER-ONTO-MORPH
Brachycephalic	Mesocephalic	Dolichocephalic
Platyrrhine	Leptorrhine	Leptorrhine
Small stature	Medium stature	Tall stature
High umbilicus		Low umbilicus
Sound teeth	Sound teeth	Badly decayed teeth
Highly susceptible to diseases of the tissues derived from mesothelium (circulatory system)	Highly susceptible to same diseases as hypo-onto-morph	Highly susceptible to diseases of the tissues derived from epithelium (central nervous system, alimentary canal)
Small face	Large face	Intermediate sized face
Short legs	Long legs	Intermediate length legs
Short arms	Long arms	Intermediate length arms
Short, flat nose with depressed root and bridge, flaring nostrils that open forward	Large, straight nose with straight bridge depressed root, nostrils open downward and slightly forward	Long, narrow nose, with high root, high bridge, nostrils open downward

These three types were previously designated Primitive, Australoid and Iberian respectively, and the present nomenclature was adopted after Dr. Hrdlicka suggested that the former names were inappropriate. The present terms do not fit entirely, as may be seen where the Meso-onto-morph has larger face, longer legs and longer arms than the Hyper-onto-morph. The Meso-onto-morph represents a form that has developed these characters to a greater extent than have the other types, whereas the Hyper-onto-morph has developed other characters to a greater extent than have the other types.

The Hypo-onto-morph is characteristic of the Filipino and Asiatic, and the Meso- and Hyper- types partake of the form of the Hypo- among these people, therefore the term Hypo-phylo-morph may be applied to the Filipino and Asiatic. Likewise the term Hyper-phylo-morph may be applied to the European, and Meso-phylo-morph to the negro, although the negro is Hyper-phylo-morphic in face, arms and legs.

THE CULTIVATION OF TISSUES IN EXTRANEOUS MEDIA AS A METHOD OF MORPHOGENETIC STUDY[1]

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During the past year we have heard much of the subject of this discussion, and the fact that tissues of the higher animals may be cultivated outside the body has been heralded in the newspapers and magazines as a notable, if not a revolutionary, scientific discovery. When we pause to consider this claim in the light of what has actually been accomplished, we find that there is danger not only of confusing in our minds several quite different things, but also that in our enthusiasm for the novelty of the method we may forget the fundamental problems to the solution of which it may be able to contribute—if used critically.

We are taught in every text book of general biology and of physiology that the organism is made up of constituent cells, which, while interdependent in the body of the organism, nevertheless have a certain autonomy. In the usual physiological discussion of death, for instance, we find the distinction drawn between the death of the organism as a whole and the death of its constituent parts. It has been known for a considerable time, that even the heart of warm blooded animals may be isolated from the body and with proper nourishment may continue to beat for many hours; more recently some very remarkable cases of the survival of organs have been reported by Carrel,[2] who has demonstrated that the vital functions of isolated organs may be kept in abeyance for days, to begin again without impairment when the organs are reëngrafted into the body of an animal.

[1] Read before the American Association of Anatomists, December 27, 1911 at Princeton, N. J. in opening the Symposium on "Tissue Culture."

[2] Jour. Exp. Med., vol. 9, 1907; ibid, vol. 12, 1910.

While the experiments made with fragments of tissue cultivated *in vitro* show that cells which are entirely isolated or are left together in small aggregates have the faculty of survival in common with whole organs, it is not this, as such, nor is it the fact that we can cultivate animal cells in extraneous media just as we can grow microörganisms, that would entitle the experiments to more than passing attention. Their importance rests, rather, upon the circumstance that they afford a means not only of observing the activities of cells when freed from the entanglements of the organism, but also of studying the conditions which influence these activities—activities which constitute morphogenetic as well as maintenance functions of cells and tissues.

As far as questions of morphogenesis are concerned, our method of investigation is but the logical outcome of the experimental embryological method of Roux, Driesch and Born, in which the isolation of embryonic parts is employed to test their function in the formation of the organism. It was a definite problem of this kind, concerning the development of the nervous system, that led me to carry the method to its extreme after the observation of normal preserved embryos and other methods of experiment had gone as far as they could and had failed to solve the problem. In order to determine whether the neuroblasts are in themselves competent to form nerve fibers, it became necessary to remove them from all sources of contamination obtaining within the body. The ubiquity of Hensen's protoplasmic bridges or Held's plasmodesms, was always liable to vitiate observations upon the growth of nerve fibers within the embryo, so that the only effective way to proceed in this particular case was to place embryonic nervous tissue in some compatible foreign medium, where it would have the chance to grow independently of its usual surroundings.

The logical process involved in an experiment of this kind affords nothing unusual. Two factors, the protoplasmic activity of embryonic nerve cells and the peripheral protoplasmic bridges, were suspected of giving rise to nerve fibers. The experiments in question eliminated the second of these factors, previous experiments having eliminated the first, and the results showed beyond

question that nerve fibers are formed only when nerve cells are present, and that they are formed as well in the absence as in the presence of the protoplasmic bridges.

There are many who would deny to this type of experiment validity in elucidating the phenomena of normal development, maintaining that the experimental conditions are too radically different from the normal to be of value in interpreting the latter. Objections of this kind are not without ground, and they have not been disregarded by those who have employed the experimental method. They have been precisely stated by Roux in his "Einleitung zum Archiv für Entwickelungsmechanik,"[3] where it is pointed out that experiments upon living organisms, particularly in cases of mutilations or of disturbance in the arrangement of parts are likely to call forth secondary regulatory processes, and it is the difficulty in distinguishing between these and the mechanisms of the direct or normal development that constitutes the danger in drawing conclusions regarding the latter from experiments such as those just described.

What, then, are the criteria by which we may judge whether experiments upon living organisms may be used in interpreting normal processes of development? In attempting to answer this question we are confronted with a more fundamental one and are forced to ask ourselves if there is actually anything peculiar in this regard in experiments upon developing organisms beyond the fact of the complexity of the material.

All 'normal' vital processes are, as we observe them, bound up with complex things which we call organisms. As soon as we institute any disturbance of the organism, whether it be by removing part of it, by stimulating one of its nerves electrically, or, for the purpose of studying its behavior, by placing it in an environment which it would not find in nature, we are bringing about 'abnormal' conditions and may be calling forth all kinds of regulatory responses. We cannot, therefore, be sure that just the same factors combine to produce the result in the 'normal' organism as in the one experimented upon. In other words,

[3] Arch. f. Entwickelungsmech., Bd. 1, 1894, pp. 19–20.

the argument against the experimental method based upon the ground that abnormal conditions arise, is quite as cogent against the simplest physiological experiment as against the most radical one in morphogenesis. And further, the same argument might be urged even against physical and chemical operations, on the ground that the exclusion of one factor released some entirely different factor which did not act in its presence. In other words, it seems clear that, as far as the logic of the experimental method is concerned, the occurrence of regulatory processes in organisms affords but a particular illustration of those circumstances known as 'Plurality of Causes,' and 'Intermixture of Effects,' which render experimental work in any field full of difficulties and pitfalls.[4]

Neither the physicist nor the chemist allow themselves, however, to be deterred from experimenting by difficulties of this kind. They do not confine themselves to the contemplation of the 'normal,' i.e., to events as they occur in nature, but they endeavor by processes of analysis to resolve natural phenomena into more elemental factors, from which, for purposes of verification, phenomena like the original may be recomposed or be represented more or less perfectly by some kind of model, according to the completeness of the knowledge gained by the analysis.

Why, then, should we, in morphology, be still so dominated by the conception of the object as it occurs in nature, the organism as a whole, which to many seems to be a sort of fetish not to be touched lest it show its displeasure by leading the offender astray? There is no real ground for maintaining this attitude. On the contrary we should endeavor to extend our experimental analysis wherever possible, recognizing that through study of the abnormal, which consists merely of those combinations of conditions and effects that do not ordinarily occur in nature, we have the best means of reaching an understanding of the normal, and that it is necessary to investigate the properties of the constituent parts of organisms before we can hope to understand them in their entirety. Because of our limited knowledge we are for the present setting ourselves an impossible task if we expect to determine with

[4] cf. J. S. Mill, Logic, 8th Ed., New York, p. 311.

certainty by means of a few experiments exactly the combination of factors involved in the normal ontogeny of any particular structure. In fact we can never 'explain' the processes of normal development with more than a certain degree of probability, until we succeed in synthesizing organisms[5] from simple known constituents or construct working models that show all of the essential activities of organisms—achievements from which we still are very far removed. Syntheses may possibly be made, however, at different stages of the analysis with components of greater or lesser complexity. Thus it may be possible to extend the remarkable experiments of H. V. Wilson,[6] who has succeeded in getting the dissociated cells of sponges and hydroids to reassemble and develop into normal organisms, so that similar methods may be applied to verify the results of analyses made by the methods of cultivation of cells and tissues outside the body.

While the foregoing considerations may have succeeded in establishing the point that the immediate object of experimental morphogenesis should be analysis, and that the 'explanation' of normal development will only come with any degree of certainty in a roundabout way, after the analysis has been made, they have not given a satisfactory answer to the query made in the beginning "What are the criteria by which we may judge whether experiments upon living organisms may be used *directly* in interpreting normal processes of development?" In fact we have almost stated that there are no criteria by which we may be absolutely certain that the same combinations of circumstances are operative in producing an effect within the untouched organism and in the one experimented upon. We can, however, extend the results of experiments directly to cover the normal processes of development provided we are reasonably sure that the circumstances of plurality of causes and intermixture of effects have been excluded, or at least that regulatory processes are not taking place. This can best be accomplished by careful comparison of what occurs in experiments with what can be observed in the normal developing organism. What we then find in the experiments

[5] cf. Mill, op. cit., p. 328.

[6] Jour. Exp. Zoöl., vol. 5, 1907; ibid, vol. 11, 1911.

under known conditions may be used to assign, with probability, definite causal connections to similar sequences of events in the organism. Such a process of reasoning is most certain in cases of self differentiation.

Satisfied that experiments upon living organisms are not by any means misleading even when abnormalities result, and that they differ from other experiments only in the degree of complexity of the conditions to be analyzed, we may now consider the purpose and the advantages of the method of tissue culture itself. In the first place, it enables us to take an essential step in the analysis of the phenomena of development into their elements in that it makes it possible to study the properties of individual cells and small masses of homogeneous tissue of almost any kind at any stage of development, whereas heretofore in our studies upon the morphogenetic properties of living cells we have been limited to the unicellular organisms, the germ cells and the blastomeres of the segmenting egg. In particular, the method of culture in small glass chambers renders amenable to direct observation a wide range of phenomena that otherwise could only be inferred by putting together observations upon preserved tissue; also it is possible by the method to subject the isolated cells and tissues to almost any changes of conditions, whereby the effect of the external medium and the interaction between cells of various kinds may be studied; or the self differentiation of any tissue may be tested with a degree of precision heretofore unattained.

The processes of development may accordingly be studied by the method with different immediate ends in view. The object may be to find out simply whether a certain tissue arises from one rudiment in the embryo or another, as was the case with the first experiments of my own upon the nerve fiber. Experiments of this class are dependent for their accuracy, as pointed out previously, upon a high degree of self differentiation in the tissues studied. On the other hand, the purpose may be to study the factors, both internal and external, which influence various processes of development such as growth, movement and tissue differentiation. In experiments of this kind all that we can expect to do is to imitate by means of known factors the conditions found

in the embryonic body, and by varying them in a known way to establish definite relations between each set of conditions and its result. By comparing the phenomena with those observable in the embryo, we may with a varying degree of probability draw conclusions regarding the causal nexus of the factors acting within the normal body. In carrying over the conclusions drawn from such experiments to the phenomena of normal occurrence we are subject, however, to all the hazards which attend experimental work in any field, and these are in proportion to the complexity of the conditions to be analyzed. That the hazard may be lessened to a minimum we must vary our experiments as much as possible in essentials, and above all must never lose sight of what can be observed in the normal organism.

In making my experiments on the development of the nerve fiber, the point that concerned me most at the outset was to establish as securely as possible the thesis that the processes of development witnessed in the artificial cultures were the counterpart of what took place within the embryo, for failing in this, the method would have been of no value for my immediate purpose. Accordingly, after finding a medium in which the embryonic tissues would live for a long period, the first thing to do was to study the activities of as many different tissues as possible.[7] In this preliminary work it was found that nearly all embryonic cells exhibit marked motility, which, as detailed observation show, is due to the movement of the hyaline ectoplasm accumulated at the borders and especially at the angles of the cells. Fine filamentous pseudopodia are there formed, and through their activity the cells change shape or move from place to place. The character and rapidity of the motion is not the same in all kinds of cells. The most independent in their movements are elements from the neural tube and the axial mesoderm, which often form a mesenchyme-like network, and the former also give rise to the long filamentous processes identical with the primitive nerve fibers of the embryo. Epidermal cells remain in sheets and frequently produce along their border a hyaline fringe of ectoplasm with pseudopodia simi-

[7] cf. Harrison, Proc. Soc. Exp. Biol. and Med., vol. 3, 1907; Jour. Exp. Zoöl., vol. 9, 1910.

lar to that formed in the healing of wounds. In contrast to this behavior, cells of the notochord and yolk-laden endoderm remain inert. These activities of course bear comparison with the processes of mesenchyme wandering, the outgrowth of the ganglion crest, the formation of nerve fibers, etc., in the body of the embryo. A still closer parallel exists between embryonic and extraembyronic processes of tissue *differentiation*, for we find that each kind of cell isolated in the culture media undergoes characteristic histogenetic changes. This is very striking in the case of striated muscle, pigment cells, and ectodermal structures such as the cuticular border and cilia, not, at present, to mention nerve fibers. These studies reveal, therefore, a high degree of self differentiation of the cells of the frog and chick embryo at the stage under consideration, and for that reason the method may be safely used for the determination of the source of tissue elements in the normal embryo. By pushing our studies back into the earlier stages of development, in all probability we shall be able to gain much further information with regard to the exact period at which potential differentiation (not immediately perceptible) takes place in each tissue.

Having established the validity of our method, the application of it to the central problem of nerve development became a matter of no difficulty. To decide which particular cells gave rise to the actual nerve fiber it was necessary merely to isolate the suspected ones, i.e., the embryonic nerve cells. The result clearly showed that the cells of the ganglionic centers, either the medullary cord or the cranial ganglia, alone produce the nerve fibers, in first instance by a process of movement, whereby the fiber is drawn out to a thread, and this is followed by a process of chemical differentiation within the protoplasmic filament, whereby neurofibrillae are laid down. There can thus be no doubt but that the substance of the axone is derived from the ganglion cell alone in the manner first described by His.

The experimental analysis of the factors which influence the movement of cells, as regards both intensity and direction, will be found to have a direct bearing upon the interpretation of normal

ontogenetic processes.[8] The study of the general conditions under which movement is possible must be carried on with reference to conditions of light, temperature and chemical constitution of the medium, including oxygen supply. In this way we shall be able to find not only the limits within which the locomotor activities may take place but also the factors which accelerate or retard them. The same problem obtains in respect to the phenomena of cell multiplication and growth, but unfortunately we here have a difficulty in distinguishing between mere movement (wandering of cells) and actual growth due to proliferation. A good beginning in this field has been made by Carrel and Burrows,[9] by Ruth[10] and by M. R. and W. H. Lewis,[11] and, especially for the phenomena of tumor growth, by Lambert and Hanes.[12]

There is another factor in the environment which has to do neither with external forces nor with the chemical composition of the medium, but yet is of great importance in its influence upon the movement of cells. I refer to the presence of some form of solid support upon which the cells may creep. The necessity of some such support seemed probable from the results of my first experiments with nervous tissue.

Experiments which I carried on during the past summer have proved the correctness of the supposition as far as the various kinds of cells in the embryos of the frog and the chick are concerned, thus affording a striking example of cellular stereotropism, a form of reaction considered by L. Loeb,[13] from his numerous experiments, to play an important part in the regenerative growth of epithelium and in the proliferation of carcinomatous tissue. The purpose was accomplished by varying the character of the solid surface offered, as well as that of the fluid media, with

[8] cf. W. Roux's studies on cytotropism and kindred phenomena, in which methods somewhat like the present ones were used. Arch. f. Entwickelungsmech., Bd. 1, 1894; ibid, Bd. 3, 1896.

[9] Jour. Exp. Med., vol. 13, 1911.

[10] Jour. Exp. Med., vol. 13, 1911.

[11] Anat. Rec., vol. 5, 1911.

[12] Jour. Exp. Med., vol. 13, 1911; ibid, vol. 14, 1911; Jour. Am. Med. Ass., vol. 56, 1911.

[13] Arch. f. Entwickelungsmech., Bd. 6, 1897; Bd. 13, 1902.

the result of showing that movement would take place upon various solids in media of different constitution, but not in the same media when the cells were deprived of solid support.

The early observations which had led me to suspect the importance of support were merely that specimens cultivated in lymph which clotted well almost always showed movement, while in those in which the lymph failed to clot this was never the case. The first experiments directed to the definite solution of this problem were made upon tissues from early frog embryos.[14] Clotting media were dispensed with, solid support was offered in the form of spider web upon which the tissues were mounted in small drops of fluid, such as defibrinated serum and inorganic salt solutions, which were varied in the different experiments; controls were made in which the tissue was suspended in hanging drops of the same media but without support. In the preparations in which the tissue is supported on a film of spider web much active movement takes place. Strands of cells are found to invest the web fibers, just as in the embryo they form the sheath to nerves, tendons and blood vessels.[15] Single cells adapt themselves to the web, assuming bipolar form on single fibers, tri- or quadripolar form when two fibers cross one another. Change of form and locomotion may be readily observed, but only when the cells are in contact with the solid support. On the other hand, the hanging drop preparations show no activities of this kind, the tissue partly disintegrating into cells which remain rounded and inactive, though differentiation within small masses of cells often takes place, especially in muscle. Apparent exceptions to the rule proved to be due to the contact of the tissue with the coverglass, upon which it was found that cells would migrate. The last named observation led to a further and more extensive set of experiments upon tissues of the chick embryo, with further variation in the solid support. In each group of experiments four different kinds of preparations were made, in which the same

[14] cf. Harrison, Science, vol. 34, 1911. Simultaneously with this Carrel and Burrows showed that cells would cling and grow upon fine silk textiles and cotton fibers. Jour. Exp. Med., vol. 14, 1911.

[15] cf. J. Loeb. Jour. Morph., vol. 8, 1893; Biol. Lect., Woods Hole (1897–8), 1899.

tissue and the same fluid medium were used but which differed from each other in the presence, or absence, or in the character, of the solid support. The specimens were mounted in the following ways:

1. In clotted hen plasma.
2. In defibrinated plasma but supported by spider web.
3. In a hanging drop of defibrinated plasma so small that the tissue remained in contact with the cover slip.
4. In a large hanging drop without contact with the cover slip or any other solid body.

In the preparations of each of the first three classes characteristic movement of cells of a number of different kinds of tissue took place, while in the fourth the tissue remained inert, except, in several cases for a slight movement upon the surface film. The experiments were varied both as to the tissue used and as to fluid medium, salt solutions being used in some cases, but the results were essentially the same as in the series with serum. The behavior of the cells with reference to the surface of the cover slip and spider web shows not only that the surface of a solid is a necessary condition but also that when the latter has a specific linear arrangement, as in the spider web, it has an action in influencing the direction of the movement, as well as upon the form and arrangement of the cells.

These facts are significant for our interpretations of the cell movements which take place in the embryo, especially those which occur in the breaking down of the mesenchyme, in the formation of the spinal and sympathetic ganglia, and in the laying down of the peripheral nerve fibers. The embryo at this stage consists largely of compact masses of cells with small spaces between. It is in these spaces that the embryonic connective tissue, nerve cells and nerve fibers move. The latter may be seen, as shown beautifully in Held's figures, clambering along the inner surface of the muscle plates or the under surface of the epidermis. The plasmic bridges or epithelial connective tissue of Held may also play an important function here, not, however, in being transformed into nerve fibers, a process the occurrence of which is dis-

proved by the experiments, but in forming a support or lattice along which the growing nerve fibers may entwine themselves.

By means of a modification of Pfeffer's method[16] beginnings have been made in the study of the action of chemical substances upon the direction of movement of the cells. Very minute capillary tubes in which the opening is drawn out to a still more minute point are first filled with some fluid the activity of which it is desired to test and are then implanted in the culture drop with the opening of the tube not far from the growing tissue. It is expected that in this way the movement or growth of cells may be directed either toward or away from the substance issuing from the mouth of the tube. The results are as yet too scattered and too inconstant to warrant any statement regarding them. One serious difficulty seems to be the slowness of the reaction of the cells. Whereas protozoa and swarm spores move very rapidly and respond quickly to the chemical environment, in the implanted tissue there is a long latent period before any movement begins, and it is very slow when it does come, so that much diffusion occurs from the mouth of the tube before any visible change in the preparation takes place.

By appropriate modifications of methods which have already been applied we may hope also to observe the response of various embryonic cells to heat, light and electric current. Again, by using media with solid support, such as fibrin, it may also be possible to test further the motor reactions of cells to one another, which have been described by Roux as taking place in fluid media.[17]

In the way first indicated we may hope to gain a very complete knowledge of the movements of embryonic cells, which are of great importance in morphogenesis. As a check to our notions thus gained, we are fortunate in having such natural objects as the fin fold of various vertebrate embryos in which similar activities may be observed in the living whole organism.[18]

[16] Ber. d. deutsch. Bot. Gesellsch., Bd. 1, 1883.

[17] Arch. f. Entwickelungsmech., Bd. 1, 1894.

[18] cf. Kölliker: Ann. d. Sci. Nat., 3 S., T. 6, 1846; Harrison: Sitz. Ber. d. niederrh. Ges. f. Nat. u. Heilkunde. Bonn, 1904; id., Anat. Rec., vol. 1, 1908; E. R. Clark: Anat. Rec., vol. 3, 1909; J. Ferguson: Biol. Bull., vol. 21, 1911.

The study of differentiation may be approached in somewhat similar ways though it will undoubtedly offer greater difficulties. The method is adapted *par excellence* to the determination of the limits of self differentiation, though it is not unreasonable to expect that we may ultimately find out much regarding the effect of the chemical content of the medium and other environmental factors upon differentiation of tissues.

Throughout the foregoing I have spoken of the work only in its relation to morphogenesis, more particularly to the processes of embryonic development. There are others to follow me who are more competent to speak with authority upon the uses of the method in the study of regeneration, wound healing and tumor growth, as well as upon the physiology of the simple tissues. One thing I should, however, like to point out, and that is, that the few data we now have afford additional evidence of the underlying identity of the processes which are concerned in normal and abnormal development and in the maintenance function of cells.

The realization of much that has just been said will depend upon improvements in technique. In order to study the reactions of living cells and tissues we must be able not only to keep them alive but also to maintain them under satisfactory physiological conditions, as regards nutrition and respiration, for long periods of time. Much remains to be accomplished, but we may expect much from the efforts of workers already in the field, who have brought so much ingenuity to bear upon the difficulties involved in their work.

FELLOWSHIP IN ANATOMY, UNIVERSITY OF TORONTO

The University of Toronto has received from Mrs. William Freeland the gift of an endowment for a fellowship in anatomy in memory of her father, the late Dr. James H. Richardson, who for many years was professor of anatomy in the Toronto School of Medicine and the University of Toronto. The fellowship is to be known as the James H. Richardson Research Fellowship in Anatomy, and is of the value of $500. It is open to graduates of recognized universities and medical colleges, and to students of the University of Toronto who have completed the third year of the medical course in that institution. The holder of the fellowship will be required to devote his entire time during the tenure of the fellowship to investigation in anatomy under the direction of the professor of anatomy in the University of Toronto.

BOOKS RECEIVED

PRACTICAL ANATOMY, The Student's Dissecting Manual, F. G. Parsons and William Wright, in two volumes. Volume I, The head and neck: the lower extremity, 468 pages, Index. Vol. II, The thorax, the abdomen, pelvis, upper extremity, 382 pages, Index. The two volumes contain 332 illustratioms, many of them in color. $2.40 per volume, 1912. Longman, Green and Company, New York.

MEMBRANE FORMATIONS FROM TISSUES TRANSPLANTED INTO ARTIFICIAL MEDIA

MARGARET REED LEWIS AND WARREN HARMON LEWIS

From the Anatomical Laboratory, Johns Hopkins University

THIRTY FIGURES

The hanging drop method was used for all the preparations and unless otherwise stated the medium used was Locke's solution or some modification of it made by varying the proportion of the salts or by adding dextrose, etc. These variations, however, do not seem to affect the character of the growth.

Cultures made from pieces of chick embryo in the various media often show growth in the form of a membrane of which there are several types found in our preparations. A very common type may be designated as the syncytial membrane (fig. 1). This membrane is found in connection with the outgrowth of cells from almost all the different organs of the chick embryo and is apparently composed of mesenchymal or connective tissue cells. We find all gradations of the syncytial formation from groups of radiating cells to a more or less continuous syncytial mass one layer thick, which spreads out over the underside of the cover slip.

A second and more interesting type of membrane occurs in the cultures from the stomach and the intestine. Almost every culture from either one of these organs gives an excellent example of such membranes (figs. 21, 22 and 23). These membranes are formed of small cells closely joined together and may be one or more layers thick near the old piece but usually spread out towards the periphery to a single layer of thin flattened cells. The cells are thicker and of less diameter near the transplanted piece, while those at edge are very thin and spread out over a greater area (figs. 2 and 6). These membranes may grow out from the entire

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periphery of the piece (fig. 5) or from two or three separate places (fig. 4) or from but a single portion of the original piece. The syncytial type of membrane or this more compact type may grow out side by side from the same piece (fig. 17). The cell boundaries can frequently be clearly distinguished in the living cultures. Treatment with silver nitrate and reduction in the direct sunlight show striking cell patterns due to the stained intercellular substance. They resemble the familiar patterns from the peritoneum (fig. 23) or from the endothelium of the blood vessels (fig. 28). The silver markings can usually be followed to the periphery of the membrane but they are heavier and more definite near the transplanted piece and can be traced onto the surface of the original piece. Shreds of mesentery attached to the intestine or pieces of the mesentery itself have never been observed to regenerate.

The edge of the membrane is usually quite irregular and seems to be amoeboid (figs. 7, 9 and 11). The cells sometimes end in long irregular processes with an enlarged fimbriated end (figs. 8 and 10). Occasionally at the margin of the membrane some of the cells are entirely detached from the membrane (fig. 16).

Some of the membranes show a few cells with holes of different size through them (fig. 19). The cells become ring-like and the nucleus is pushed to one side. These holes may pass completely through the cell and are bordered by a granular area, which turns brown with the silver nitrate method (fig. 19). In other membranes an entire cell or two may disintegrate and leave the nucleus in the space (fig. 18). Sometimes patterns which resemble the pseudo-stomata occur among the cells (fig. 20). Sections through the membranes growing out from the intestine show clearly that these membranes are endodermal in origin.

Cultures from pieces of the allantois (fig. 30) almost always give rise to growth in the form of a membrane very much like that from the intestine. Pieces of the amnion (fig. 29), the yolk-sac (fig. 28) and in a few cases the kidney (fig. 26), give rise to a membrane somewhat similar in character and which also shows the silver markings.

Some of the cultures especially those from pieces of the yolk-sac give rise to a very characteristic membrane similar to the above

except that each cell contains a large clear vacuole or globule close to the nucleus (fig. 27). Usually this vacuole is filled in with granules in the preparations made with silver nitrate, but it is plainly to be seen in the living cultures or in preparations made with osmic acid.

Occasionally there are two membranes, one growing out under the other. In this case one of the membranes may be the syncytial, and the other the mesothelial type, or one may be large cells as in figs 15 and 16, or in some cases the type of cells may be the same but of a different size or shape.

Cultures from pieces of heart sometimes give rise to a membrane quite different in character from these. The cells are several times the size of those in the above membrane. They contain one and sometimes two large nuclei near which there is usually a heavier granule deposit in the stained preparations. The cells may be closely packed together (fig. 14) or they may show varying degrees of separation with protoplasmic bridges of different sizes connecting the cells (figs. 12 and 13).

A fourth and perhaps the most interesting form of membrane is derived from the kidney tubules. Pieces of kidney were teased in the culture preparations so that broken pieces of tubules are scattered about or are attached to the edge of the transplanted piece. After twenty-four to forty-eight hours the connective tissue cells wander away and leave the tubules plainly to be seen. Occasionally both in the plasma (fig. 25) and in the saline media (fig. 24) there arises a strange irregular membrane which spreads out from the broken end of the tubule. The cells become flattened and irregular and form an extensive membrane. They grow wildly as though some restraining influence, which normally kept them in order, had been removed.

From these observations it seems that the growth of tissues of the chick embryo transplanted into artificial media may as in these membranes retain their specific differentiation and do not return to their embryonic type.

PLATE 1

EXPLANATION OF FIGURES

1 Syncytial membrane from intestine of an eight-day chick embryo in Locke + 0.25 per cent dextrose. Iron hematoxylin. × 650.

2 Small endodermal membrane showing silver markings from intestine in Locke + 0.3 per cent dextrose + 0.08 urea. Silver nitrate and Ehrlich's hematoxylin. × 210.

3–5 Endodermal membranes from stomach and intestine in Locke + 0.25 per cent dextrose. Silver nitrate and Ehrlich's hematoxylin. × 120.

6 Endodermal membrane from intestine in Locke + 0.25 per cent dextrose + 50 per cent chicken bouillon. Iron hematoxylin. × 210.

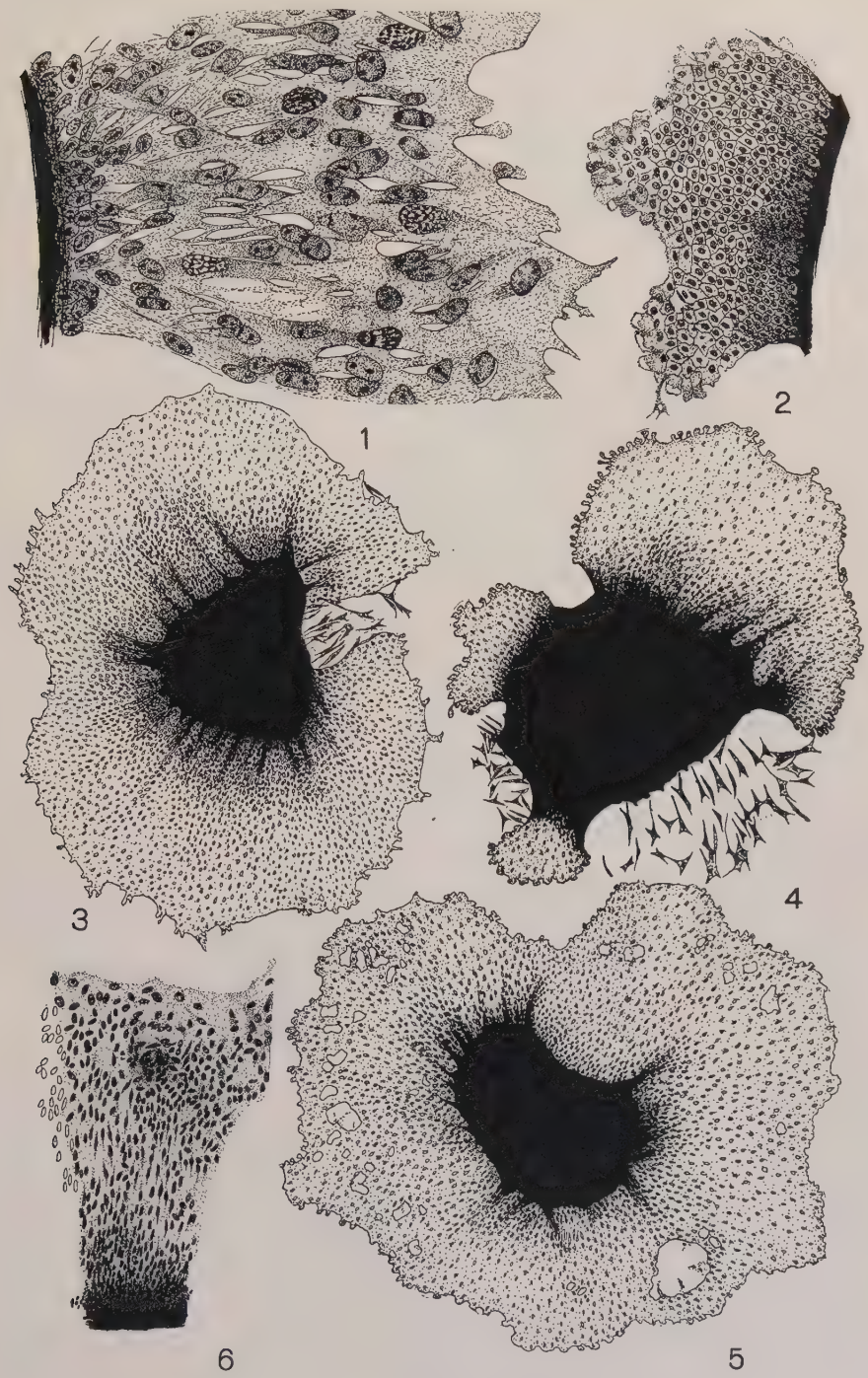
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PLATE 2

EXPLANATION OF FIGURES

7–11 Types of cells along the edge of some of the endodermal membranes. × 950.

12 Cells loosely joined together with protoplasmic bridges from heart of six-day chick embryo in Locke + 0.25 per cent dextrose. Silver nitrate and Ehrlich's hematoxylin. × 950.

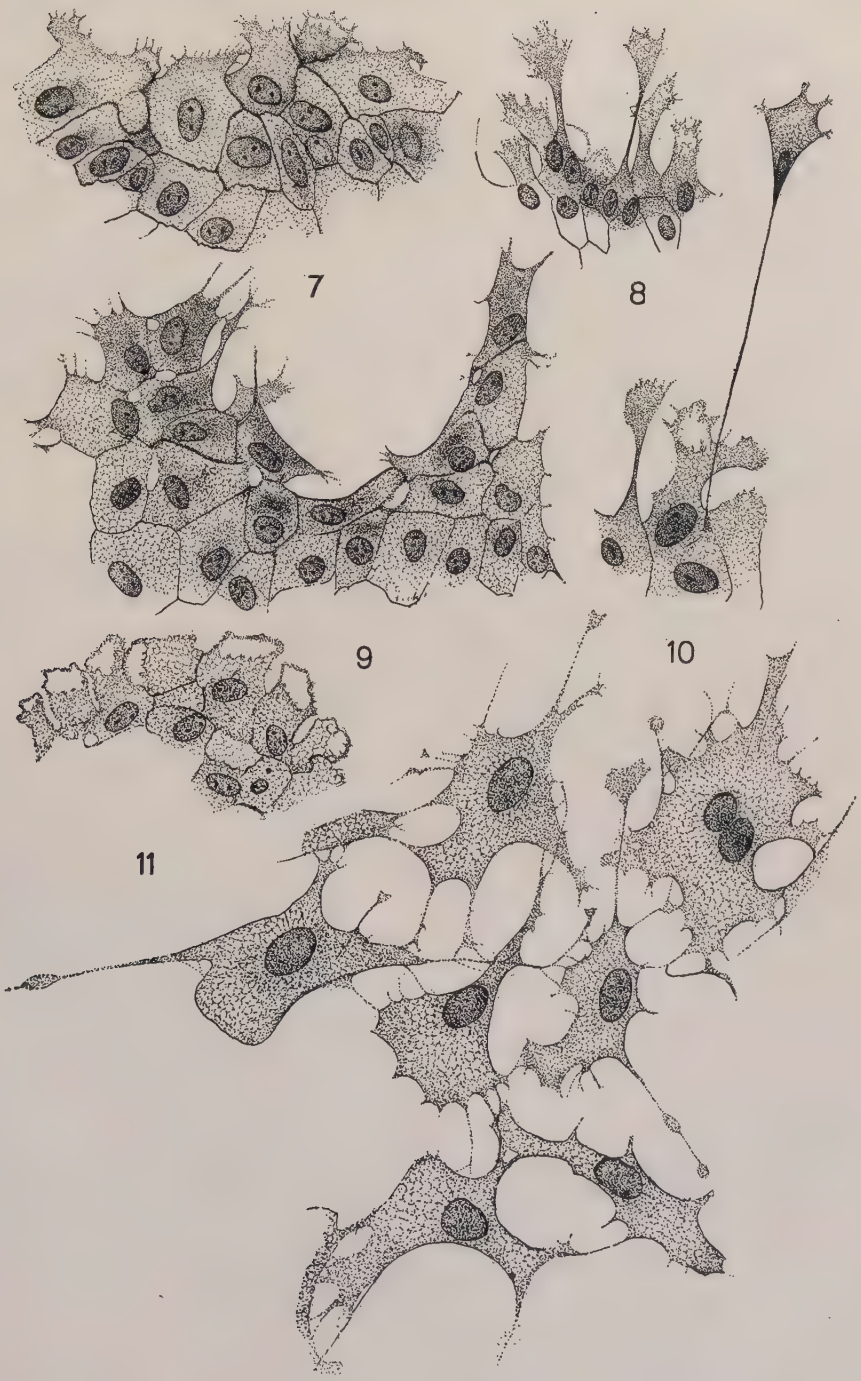
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PLATE 3

EXPLANATION OF FIGURES

13 Cells from same membrane as fig. 12, more closely joined together. × 950.

14 Cells from same membrane as figs. 12 and 13. Cells close together. × 950.

15–16 A double membrane from intestine of six-day chick embryo in Locke + 0.25 per cent dextrose. Fig. 15, focus near cover slip. Fig. 16, focus below. Silver nitrate and Ehrlich's hematoxylin. × 210.

17 Syncytial membrane adjoining endodermal membrane. Iron hematoxylin. × 950.

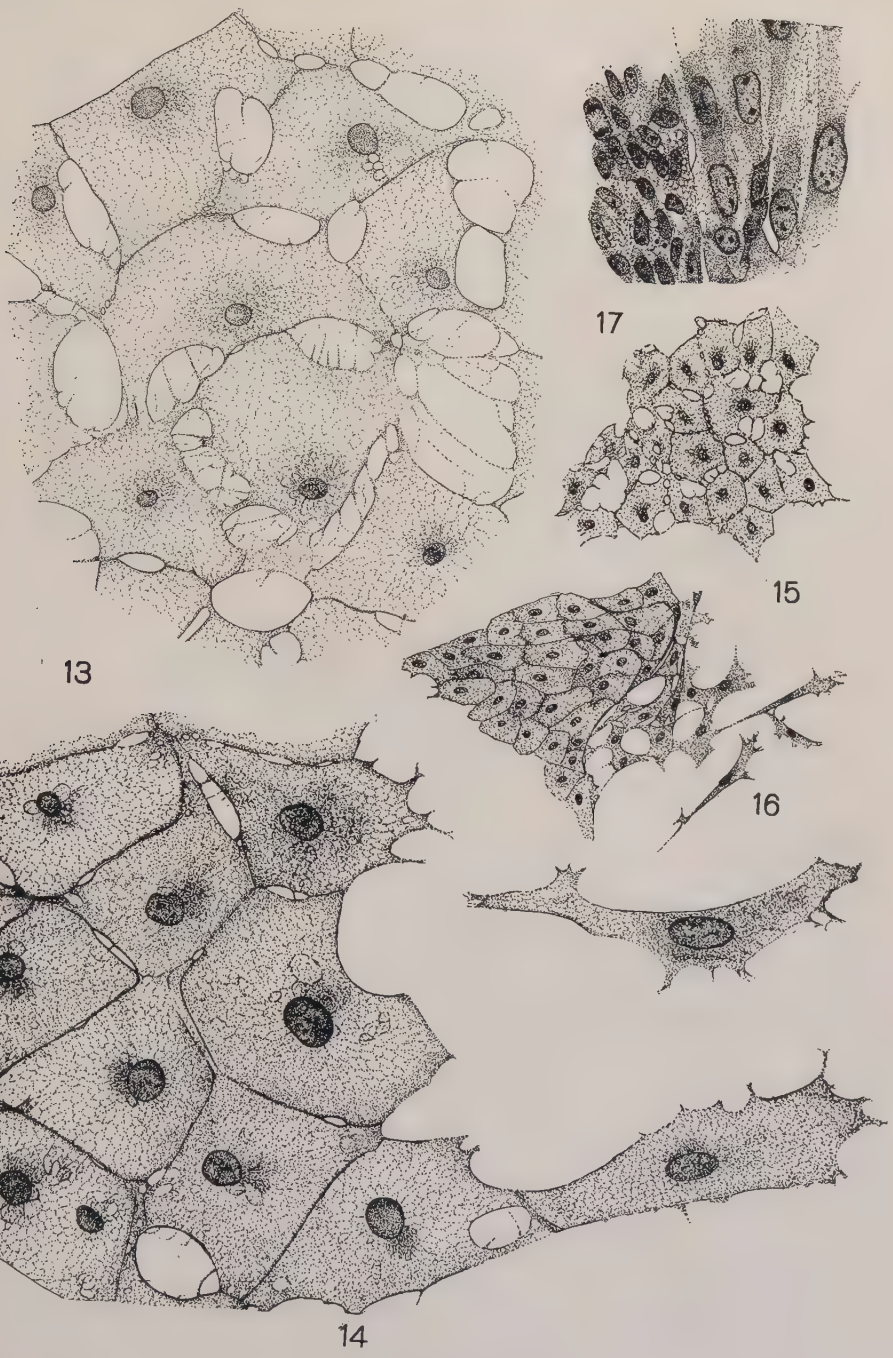
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PLATE 4

EXPLANATION OF FIGURES

18 Cells disintegrated leaving space in membrane from intestine of ten-day chick embryo in Locke + 0.25 pe cent dextrose. Silver nitrate and Ehrlich's hematoxylin. × 950.

19 Holes in cells of the membrane from intestine of an eight-day chick embryo in Locke +0.25 per cent dextrose. Silver nitrate and Ehrlich's hematoxylin. × 950.

20 Pattern like pseudo-stomata. Intestine ten-day chick embryo in Locke + 0.3 per cent dextrose. × 950.

21-23. Patterns from pieces of intestine in Locke + 0.25 per cent dextrose. Silver nitrate and Ehrlich's hematoxylin. × 950.

24 Membrane from kidney tubule of ten-day chick embryo in Locke + 0.25 per cent dextrose. × 210.

25 Membrane from kidney tubule of ten-day chick in plasma. × 210.

26 Pattern of cells in membrane from kidney of seven-day chick embryo in Locke + 0.25 per cent dextrose. × 950.

27 Membrane in which each cell has a large vacuole adjoining a syncytial membrane, from intestine of six-day chick embryo in Locke + 0.25 per cent dextrose. × 950.

28 Pattern of cells in membrane from yolk-sac of four-day chick embryo in Locke + 0.25 per cent dextrose. × 950.

29 Pattern of cells in membrane from amnion of seven-day chick embryo in Locke + 0.25 per cent dextrose. × 950.

30 Pattern of cells in membrane from allantois of four-day chick embryo in Locke + 0.25 per cent dextrose. × 950.

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THE CULTIVATION OF CHICK TISSUES IN MEDIA OF KNOWN CHEMICAL CONSTITUTION

WARREN H. LEWIS AND MARGARET REED LEWIS

From the Anatomical Laboratory, Johns Hopkins University

In the June number of The Anatomical Record we gave an extensive table of over eighty combinationsl of NaCl, $CaCl_2$, KCl and $NaHCO_3$ and water to form culture media for the growth of embryonic chick tissues. It was possible to obtain growth in such media in which either the $CaCl_2$ or the KCl or the $NaHCO_3$ was omitted but not when the NaCl was left out. The most extensive growth both in quantity and duration was with a media of the following constitution: NaCl 1 to 1.5 per cent, $CaCl_2$ 0.025 per cent, KCl 0.042 per cent, $NaHCO_3$ 0.02 per cent. Such growths continued for several days but were never as extensive either in quantity or duration as growths in the plasma media.

The tissues from the younger chick embryos (four to eight days) seem to grow better than the older ones. Tests were made with liver, heart, intestine, stomach, spleen, adrenal, kidney, limb buds, thyroid, lung, eye, brain, spinal cord, etc., though not exhaustively. All these various tissues showed some form of growth usually as radiations, reticular formations, migrations, membranes or nerve processes. There is a certain uniformity in the outgrowths as might be expected from tissues containing a similar vascular and connective tissue framework. A portion of the outgrowth is undoubtedly merely a wandering out of the (mesenchymal ?) cells into the surrounding medium. The presence of mitotic figures, the formation of membranes and the outgrowth of nerve fibers show that we are also dealing with real growth. It is often difficult or impossible at present to determine exactly the identity of the various cells that grow out from the transplanted pieces. Nerve fibers are usually easy to recognize in both the living and stained preparations. The membranes

from the kidney tubules one may also be certain of identifying when they are continuous with the tubules. The (mesenchymal ?) outgrowths even from the same organ vary considerably yet there is a general uniformity. There are several types of cells in these outgrowths and we are uncertain as to their identity. The most common type is probably mesenchymal or connective tissue but even here uncertainty exists. So that one of the most urgent problems in connection with this work is the identification of these various outgrowing cells.

We have continued the work on the cultivation of sympathetic nerve fibers from the intestine and stomach of the chick and a short communication on the subject appeared in the January number of The Anatomical Record. Such sympathetic nerve fibers seem to come from cells in the small ganglia of the plexuses in the walls of the intestine. These nerves grow readily in various saline solutions with or without small amounts of dextrose. The outgrowth of the nerve process begins earlier and progresses more rapidly in saline solutions than in plasma or lymph and the extent of growth is apparently equal to that in either of these media. The nerves show the characteristic active amoeboid endings usually sending out long thin processes which often anastomose with other nerve ends or fibers. The fibers themselves are richly supplied with fine lateral branches of varying sizes. These branches are often quite active, extending out, retracting, pushing out again in the same or another place and often forming anastomoses with neighboring fibers. There are also numerous anastomoses between fibers by means of larger branches. The neurofibrils in stained specimens can actually be traced through these communicating branches from one main fiber to another.

In these cultures the sympathetic fibers often begin to grow out within ten hours after the cultures are made and at twenty-four hours the process is well advanced and there is often a dense jungle of anastomosing fibers. Degeneration usually begins by the forty-eighth hour. The fibers extend out to a length of from 1 to 1.2 mm. and may grow as rapidly as 1μ per minute. They stain readily in Heidenhain's iron-haematoxylin and show the neurofibrillae. If the amount of extraction is just right the fibrillae

appear to be granular in structure but the real significance of the granules is uncertain. The great similarity in reaction to chromatin suggests a relationship to this nuclear material. The nodosities or varicosities scattered along the nerve fibers react in the same manner and are composed of similar neurogranules.

Similar cultures from the spinal cord and medulla in these solutions sometimes give pictures almost identical to those seen in the outgrowing sympathetic nerve fibers. The characteristic amoeboid nerve endings, the rich supply of fine lateral branches as well as larger ones, the branching and anastomosing of large and small fibers from the nerve endings and main stems are all very similar in the two kinds of fibers. The nodosities or varicosities so characteristic of the sympathetic fibers are also present but they are not so frequent nor so large. As with the sympathetics during the first twenty-four hours after the culture is made the outgrowth is often very extensive and we frequently find a dense jungle of anastomosing fibers. Not all cultures of nerves from the central nervous system however resemble so closely the sympathetic nerves for the outgrowths of nerves differ considerably in character with the age of the embryo used and the region from which the piece is taken. We hope in the near future to give a much more extended account of these outgrowths.

One of the most suggestive features in connection with these nerve outgrowths is the rich supply of very fine lateral branches, many of them so fine that they can hardly be seen with the oil immersion and No. 8 or 12 ocular, and even then as one follows them to the end the impression is left that had we greater magnification and finer definition it would be possible to follow them still farther.

From the nerve ends many fine branches radiate out in all directions at various angles but along the nerve stem they usually run off at right angles. Sometimes they are opposite one another but usually they alternate, and in fixed specimens owing to their adhesion to the coverslip they may give to the main stem through shrinkage a slightly zig-zag course. Their extreme motility suggests that the old theory of sleep by Duval and Cajal through retraction of the gemmules on the dendrites has in these fine

branches some basis of fact. Perhaps more important still may be their bearing upon the establishment of reflex and cerebration paths. It is not unlikely but that in the much more favorable environment of their normal position in the central nervous system their branching would be richer and longer and instead of being reduced to one plane as in cultures where they creep along on the underside of the coverslip, they would extend out in all directions not only from near the ends of the axones but from the dendrites as well. We have in these plastic branches which readily form anastomoses that are more or less permanent or merely transitory, a device for the formation of paths of varying degrees of strength and permanence. Through constant use for example (which means more or less continuous metabolic activity) we can imagine that the size and richness of the anastomoses they form might be such as to remain throughout life, even though in after years such paths are less frequently used. Again it is not difficult to picture paths established in which the number and size of the anastomoses vary greatly and correspondingly the permanence would likewise vary greatly or they may even entirely disappear. Parallel with this, for example, we have reflexes and memories of varying degrees of permanence.

This importance of these active branches is only a suggestion, for the jump from the conditions of the cultures in salt solutions to those existing in the adult human brain is a gigantic one.

Such active processes on the ends of the outgrowing peripheral nerves suggest a method by which it is possible to picture how nerves become connected with their end organs. The active extension outward in all directions of these fine branches, creeping along for example on mesenchymal cells or their processes, would give abundant opportunity for many of them at least to reach end organs, and having done so such connection would remain permanent and increase in strength through increased metabolic activity. The unsuccessful branches on the other hand would be retracted. It is very surprising the distance to which these fine branches may extend in the salt solutions and in the better environment of the body this would probably be much greater.

In regard to the cultivation of tissues in media of known chemical constitution, we can sum up the work very briefly. Aside from various combinations of the salts mentioned above and of these salts with various sugars we have reported very briefly our success with certain combination of the amino-acids and polypeptids in conjunction with the late Dr. Koelker who has supplied us with many of these synthetic preparations. While some of the growths are better than those in simple saline solutions, they are by no means equal to those in plasma or lymph either in extent or duration.

Combinations with chicken and beef bouillon are not much if any better than the Locke's solution.

We have tried cultivation in combinations of Locke's solution with the filtered and boiled digestive products of liver, muscle and brain. Growth took place in some of these media, such growths, however, were no greater than we have obtained in Locke's solution.

Our aim here is of course to find a medium that will permit of the continuous growth in large quantities of the tissues. Perhaps we may be able even to obtain selective media and secure pure cultures of cells of one type, as the liver, heart muscle or adrenal cell. One need not dwell upon the importance of such a method for the study of the properties of tissues and cells both from an anatomical and chemical view-point.

SOME PRINCIPLES OF ORGANIZATION TO SECURE PRACTICAL RESULTS IN ANATOMICAL TEACHING[1]

H. McE. KNOWER

Cincinnati, Ohio

The general adoption of an affiliation of medical schools with universities was, doubtless, the most important step toward securing a course properly correlated with modern developments in all departments of science and educational method.

Unfortunately however, the readjustments demanded by this change in teaching, from independence frequently extreme, to the new system, have been made more difficult by the almost radical transformations which have been at work in the sciences fundamental to medicine.

Physiology and pathology, and even zoology, are becoming more chemical; pathology, pharmacology, bacteriology, and zoology more experimental. Human anatomy has adopted a more embryological point of view, with its comparative basis more experimental and dynamical. The growth of these new lines of work alone, in either one of the fields of anatomy, physiology, pathology, etc., has been so rapid as to fill many special journals (some created to hold this new matter), and

[1] In printing this statement of what seem to be the most urgent needs of anatomy at the present time, we must acknowledge our debt to Prof. F. P. Mall of the Johns Hopkins Medical School for his frequent insistence upon many of the essentials of a satisfactory organization. It is especially due to the convincing nature of Dr. Mall's argument and practical demonstration that there has been a decided increase in the number of anatomical laboratories which have been reorganized, in the last few years. These are now administered in a manner to take account of the constructive evolutionary changes which have affected the development of modern medical education. His articles in the Johns Hopkins Hospital Bulletin, Anatomical Record, etc., should be studied by everyone interested in modern anatomical work. The present plea, however, though based on the same scientific principles, aims to formulate comprehensively, in one place, certain essentials which still demand action (see also Report of the Committee of One Hundred on a Standard Curriculum for Medical Colleges, under the direction of the Council on Medical Education of the American Medical Association, 1909).

This statement was delivered in an address before the Ohio Association of Medical Teachers, December 26, 1911, at Columbus, Ohio.

to overtax the capacity of any one man who attempts to teach that branch. On the other hand, the practical applications of the results so recently attained in these sciences; and the adoption, in the more strictly medical subjects, of similar scientific methods have emphasized the necessity of the students receiving proper premedical training, before being allowed to enter the medical school.

It is in the university laboratories that the fundamental work in chemistry, comparative anatomy and embryology, and most of the experimental research in biology has been done. Every provision is therefore already made for these subjects in the college, while the entire medical curriculum is disturbed by forcing such work into its four years. The student then begins to lay the foundation at a time when he cannot devote sufficient attention to it to make it of practical use; and, at the same time, he is constantly handicapped in the effort to assimilate the medical studies normal to the first year. The university here helps us with its wealth of facilities. by furnishing our students the opportunity of securing, in advance, an adequate foundation in the scientific and other studies needed to fit them for the medical courses. So we no sooner come to appreciate the need, than a remedy is at hand! It should, however, be evident that the university departments have been too busy meeting the insistent demands of various groups of academic students, under new conditions, to have been able to plan especially for the premedical students; though here and there this attempt has been made.

PREMEDICAL REQUIREMENTS

Of the two *external adjustments*, then, which are most urgently demanded for the improvement of our anatomical teaching, the change in the character and method of holding state board examinations, which we cannot here touch upon, seems secondary to this matter of premedical requirements.

In spite of frequent discussion and laudable action, there is still a serious defect in our method of prescribing these preliminary requirements. It is not enough to make a general demand for one year's or two years' college work in biology, chemistry, physics, and foreign languages. This has already been done by many medical schools which have read the signs of the times. A little inquiry as to what college teachers in these fundamental subjects (as for instance biology), understand by a year's work, will make it clear, that there is considerable lack of agreement among them. Therefore, the only way our admission committees can secure an adequate preparation for their prospective students, is to come to an agreement as to the essentials to be insisted upon. While I would be the last to wish to dictate to the zoologist what his year's course should be; I believe the anatomists, physiologists and bacteriologists in the medical schools have the responsibility of initiating action. They must bring about an arrangement which shall permit sufficient election to enable their students to obtain in the college, at least these essentials agreed upon.

We must soon set down, for the subjects within the scope of biology, a minimum program, defining the amount of botany, parasitology, cytology, comparative anatomy, histology and embryology, etc., which anatomists agree must be required in addition to what the zoologists and botanists are offering in their elementary general courses. The biologist will know how to assist in this, and I have a confidence that he will aid us in many ways. For one thing, the course in mammalian histology now given in a number of zoological departments might be discontinued. Time would then be gained for courses of a more comparative and fundamental nature, and the anatomical laboratory could readily take care of academic students who require special histology. On the other hand, the elementry vertebrate embryology should be given in the zoological laboratory as a premedical course.

It may be possible to arrange such a program for a year's premedical work, taken with physics, chemistry and languages; but it does not require much knowledge of the details and of conditions to predict that the better students and most reasonable teachers will find it advisable to allow two years to such preparation. This will be an actual economy of time in advancing the medical work, since very few students are equal to securing a satisfactory grasp of anatomy and physiology, as understood today, without such a foundation. Besides, more time is required for the subjects of the first two medical years in proportion to the lack of preparation.

When a similar plan shall have beem worked out for chemistry, physiology and bacteriology, and expressed definitely in the statement of admission requirements, our students will come to us prepared in a practical manner. They will bring not only the general knowledge necessarily a part of the college course, but an additional special equipment, properly selected to aid them in their medical studies.

This reform can only be brought about, safely, by those teachers of the medical schools who keep themselves informed of the actual status of the fundamental sciences affecting their work. Such men can view the problem from this standpoint, as well as from the more technical side of their special department. It might be well to entrust the adjustments of the premedical schedule to a representative committee of the American Association of Anatomists.

ORGANIZATION OF THE STAFF

This brings me to the first consideration of *internal organization* affecting anatomy: the organization of the staff.

It is generally conceded, now, that the professor of anatomy should be relieved of the necessity of practicing. He should have had a broad educational experience in the sciences underlying medicine, to understand preliminary requirements; and should have worked with medical students and with medical teachers of various sort for several years, to insure a good perspective. He should be carrying on scientific

research and thus be, to some extent, an authority in his subject, while its technique should be fully at his command.

The student comes into contact with the special problems of the human body and the method of attack first in the anatomical laboratory. It is hence important that he shall here meet an organization which will determine his attitude in a desirable manner from the start. The entire anatomical staff should therefore be selected with reference to the same principles which have influenced the appointment of the professor. Though it may be vain to expect students to accomplish much, if anything, in research, this spirit and activity must be evident in the staff and must reach the students. Doubtless there will be slight disagreement as to the wisdom of such preliminaries; but it will still be necessary to organize this department further than is generally accomplished, to get the right results.

I wish now to raise the question, which I feel it is dangerous to neglect: Are we properly following up our act of freeing the professor from outside work by supplying him with enough technical assistance, clerical, and janitor service, in addition to material facilities, to enable him to carry out satisfactorily a practical method of instruction and to develop something new and useful in his department?

An often extravagant supply of apparatus, models, museum specimens and other accessories may be granted readily; but much of this can be shown to be of secondary importance, to what can be secured for the anatomical department by expenditure in conserving the energy of the staff for scientific research and teaching. When a medical school is spending several thousands of dollars for teachers' salaries, to secure a scientific system, it would surely be very wasteful to refuse a few hundred for technical aid, to permit a fair trial of this system in a satisfactory form.

It is the duty of the school to provide an effective mechanism to avoid this serious danger. It is also the plain responsibility of the professor, as far as it lies in his power, to see that the work is so arranged that each member of his teaching staff is protected from unreasonable demands; so that opportunity is left for development as an anatomist in research as well as in teaching. I am, however, fully alive to the importance of a young man seeking considerable responsibility, since it develops power and makes promotion possible.

Even when the college has provided a wealth of equipment, we frequently see some valuable member of the faculty overburdened with departmental routine or committee work, etc., to the injury of his professional growth and influence. The dean is generally a conspicuous example of this injustice in being forced to perform the functions of a clerk. I know of more than one department of anatomy in well known universities which lack a technician, and where the teachers are forced to carry the burden of this routine.

THE ARRANGEMENT OF COURSES

Having thus freed the staff from unreasonable demands and furnished students who have provided themselves with sufficient mental equipment to enable them to profit by a course in anatomy, it is now possible to arrange a medical schedule which shall honestly give up the first two years to gaining acquaintance with the body and its functions, its special chemistry, pathology, and bacteriology, while the more strictly medical studies are reserved for the remaining years. Let us see that this opportunity to give the students time enough to think is not stolen by another overcrowding of the first two years.

It is important to plead strenuously for an allotment of *plenty of time to permit the first year's students to read and think some.* They should then pursue their work in gross and microscopic anatomy, and in physiology, so that the bulk of the work is finished by the middle of the second year. The last half of the second year should not however be given up entirely by the anatomist, but should be devoted to a reconstructive study of the body through sections and special dissections. This is the best time to use models, and for the student to work out topographical and other relations, which he has seen in another way in the course of his dissection. All through the two years, possibly to especial advantage in this connection, the embryological aspects of structures should be insisted upon. Many unfinished topics suggested to the student during his course may be now completed. Special work in anatomy at just this time, where it involves some orginality of effort is, I believe, apt to be of more value than similar efforts in post-graduate years.

It is possible that a still greater concentration is desirable (though I do not believe this because it would cut down the time for outside reading), by which histology and dissecting would be mostly finished by the end of the first year. This would probably mean at least half of all work days for about thirty weeks for gross anatomy, and three half days a week in addition, for the same period, for histology. There may be some advantages in this plan, and time enough can be secured for it, if the schedule shall have been first cleared of studies which should be taken in the premedical years. It will still be necessary to reserve a part of the second year for the important final work in anatomy just outlined; and, since this reconstructive and topographical work is no longer based chiefly on dissection, but requires special material and special instruction, it must be understood that a larger teaching staff will be demanded than is required where there is not this need of managing two different kinds of courses at the same time.

It is more economical to arrange the first and second year dissection for the same hours so that all members of the staff, who are not teaching histology, may be available for this work. In the spring the dissecting can then be stopped and the staff divided to care for the two classes in separate groups; the first year's class in neurology, the sense organs, etc., and the second year in topographical and special anatomy.

In this connection we must insist upon histology being given under the same roof and by members of the same staff as the work in gross anatomy. It is no novel idea, that the relations of these two aspects of anatomy are so intimate as to positively demand this arrangement. A proper correlation of courses is then possible. Adequate conditions to encourage research, or indeed any special or advanced study can not be obtained, otherwise, without very considerable additional expenditure, by those members of the staff who are working in gross anatomy. Even where there are two separate departments, one for gross anatomy and another for histology and embryology, it is most important that the essential unity of the two should be recognized in every way. Much remains to be done in more than one prominent department of anatomy toward maintaining such reciprocal relations, as the writer knows from personal observation.

The very great advantages of a rotation by members of the anatomical staff in teaching the various divisions of the subject have been uniquely demonstrated by Professor Mall. It is worthy of a more extensive adoption in other laboratories. He has thus encouraged his staff to escape the monotony of teaching only one side of the subject from year to year. This exchange of responsibilities is not compulsory, but those of the staff who undertake it may thus better fit themselves for their work, either in teaching or in research, by the wider experience. They secure a better understanding of the needs of the various courses conducted by the anatomical department, and these courses profit greatly by the new ideas and methods thus brought in. The idea is carried still further when there is a sufficiently large staff, by encouraging students to arrange their work so that they may profit by what can be obtained from each instructor.

AN ORGANIZED PLAN OF TEACHING

We now come to the more intimate relations of staff and students, and the organization of the method of teaching; the plan of attack to be agreed upon by the staff.

The didactic method

On the one hand is the more antiquated didactic method (Teaching by Precept); with more or less able lectures, numerous formal quizzes, and a dissecting room or laboratory run by demonstrators who often, indeed, expend much effort to make sure that the students are *shown* as much as possible even to the extent of interfering with their personal work. In some places this method is carried out with almost military precision. The students might be said to be spoon-fed. They become good grinds or listless imitators, and often know their 'lessons' surprisingly well. They strive to subordinate their ideas and opinions under the weight of the authority so impressively, if not dogmatically forced upon them. This method seems the easiest for the professor, whose

lecture is the main responsibility. The dissecting room is looked on, chiefly, as a place to demonstrate and illustrate lectures and what is to be found in the more concise text-books. It soon becomes a disorderly, if not dirty place, where only a few students carry through fair dissections. Many of our best medical men have learned much anatomy in this way; but the majority of such precept-bred students are still taught quite elementary facts and relations of anatomy in the upper years. This is because of the conviction of the professors of the applied branches that the average man has not only forgotten his lesson but, what is worse, can not be trusted to look it up for himself. They expect the students to be dependent and inefficient in dealing with the body practically. Hence the veriest *A*, *B*, *C*'s of anatomy are rehearsed in many text-books of surgical anatomy, painstakingly redescribed in considerable detail, and illustrated with many figures already familiar in anatomies, or far better in modern atlasses.

This method of teaching, then, not only fails to insure the memory of many details which one may forget after following any method; but must be criticised more severely, in as far as it does not furnish a commonsensed and ready grasp of essentials. A method which may give a fair literary knowledge but fails, as this does, to develop a practical ability to handle the body, as well as to talk about it sensibly, is defective in a serious place. Of course the above argument applies to histology as well as to gross anatomy.

The scientific laboratory method

Opposed to the type of anatomical teaching just discussed is the other extreme; organization for Education through Guidance, which makes every possible arrangement to permit and to encourage self-education in the student. No one who understands modern laboratory teaching, which keeps ahead of text books as here planned, can fail to realize that this method is much more exacting on the staff than lecturing, or formal quizzes, since it involves so much individual guidance.

In advocating this point of view now steadily gaining ground, for attacking the problem of anatomical teaching, I believe it is necessary to explain that it does not seem to me reasonable to take an extreme position. We must not expect even our most carefully selected students to learn human anatomy by themselves (!) in the course of two years, while at the same time trying to correlate this work with other unfamiliar subjects. I know there is frequently this misconception of this method, but the guidance is an important side of the application of the plan. 'Self-Education under Guidance,' as Professor Mall so well puts it after von Baer, is quite removed from this extreme. *The guidance is to be accomplished by an entire system, all parts of which coöperate to produce the desired result.* The students will need assistance, and much thought and effort will be demanded of the staff to teach them, individually the best methods of working and studying independently. Again I must insist that ample time is essential. A sufficient number of experienced

men must be supplied as teachers, and I, for one, believe that the beginners should come into close contact with the professor, as well as with his assistants.

The emphasis is to be thrown on the student's own problem. And this problem should be so to study his material as to build up daily, from his personal findings in the laboratory and in books, a conception of how the elements which he has worked out in detail are not simply to be identified and memorized, but to be combined into a whole. He is taught to associate the elements of structure into functional groups. The chief groups which act together to perform the functions of any part are arranged into certain primary divisions; and since much of the history of this adjustment of anatomical structure to physiological demands is written in embryology, the student's premedical studies should be of great value from the start. Thus, the memorizing of the contents of the triangles of the neck, the layers of a hernia, the names of the branches of an artery or nerve, or the attachments of muscles, evidently, follows as of secondary importance to the first effort; to understand the more fundamental grouping adaptive to function, with the aid of embrology. There is not time to explain this more fully here, but this will be done in another place.

The student must be led to learn a method of work with his specimens and books, which is practical and adapted to his particular needs; and he must secure some considerable mastery of the subject and self-reliance in dealing with it to be passable. If he will not or can not thus secure command, he must drop out, for he is not yet ready to even begin a medical career.

The laboratory must furnish up-to-date facilities, good material of sufficient variety, and enough instruction to insure a modern outlook on anatomy, in advance of the text-books and with a strong physiological bias whenever possible. The instruction will generally be beinformal and suggestive, more by questions than by assertions; the plan being to awaken critical judgment, and to explain to the student the value of following things up for himself in the literature and by the laboratory method of practical work. The student's own efforts and results must be constantly in the foreground; and while lectures or demonstrations are certainly of great advantage, even required at times, they become actually harmful if they are allowed to detract from this stress upon his responsibility. The student is not to be left to, drift,' or to carry on 'research' for himself; but he must be given a hand only when in real need. Modern text-books and atlases are insisted on. Dissections are expected to show every possible detail, and the student is advised to keep records through notes and drawings. There is an effort to attain a high degree of completion in the work, so as to permit of a satisfactory reconstruction of the part as suggested above.

Students, who have experienced both methods of teaching, make the rather enthusiastic claim that, freshmen guided by this laboratory system make as good dissections as demonstrators following the old didactic course. It is astonishing to observe the effect of this method

in transforming an average, rather unsystematic and inefficient student who lends himself to it, into one with a commonsensed plan of work and a reasonable knowledge and practical ability to handle any part of the body intelligently. It would seem that this practical grasp and self-reliant poise is the best result of the course. The best products of the system may not be distinguished by glibness, but their knowledge is authoritative and usable.

Some able anatomists still adhere, from choice, to the method which I have characterized as teaching by precept. It will rest with those who have an opportunity to examine their students to judge how practical is the result. In defense of certain other teachers who feel obliged to use the didactic method, it should be made clear to those responsible, as faculty, president and trustees, that this is often encouraged by an inadequate support of the anatomical department. It requires a suitable staff, properly organized and supported, to carry through a thoroughly scientific laboratory system. If one man is to teach fifty students alone, or with ill-trained assistance, he may be forced to revert to the 'lecture habit' and various other wholesale devices.

AN ORGANIZED ENVIRONMENT

To accomplish anything by a practical laboratory method, it is essential to establish a correct environment. So, in demanding modern facilities for the anatomical department, it should be pointed out, that it is in this laboratory that the student may first be profitably brought into contact with some aspects of the science of medicine, and of fundamental principles affecting its practice not generally emphasized in dissecting rooms. From this point of view, the importance of devoting considerable attention to planning the rooms and the furnishings, as well as the method of work, will be evident.

A scrupulously clean, and as far as possible aseptic anatomical laboratory, in a way like a surgical operating room, with good ventilation and light, and plenty of hot water and soap, and individual paper towels for the hands, is a good object lesson in hygiene. It is also a good preparation for more dangerous work in pathology and infectious diseases, and for preventive medicine. It would be highly desirable to create an even artistic atmosphere here, as far as possible. Insistence upon businesslike conduct, and orderly habits finds a ready response from the student, and a natural place in this laboratory; and a thorough scientific method in handling the body here, is the best training in carefulness and success in dealing with the patient later. Finally, insistence on a correct standard of scientific and medical ethics in this environment is a valuable introduction to an elevated conception of the profession.

BOOKS RECEIVED

LEHRBUCH DER ENTWICKLUNGSGESCHICHTE. Dr. Robert Bonnet, 486 pages, Index, 377 Illustrations. Published by Verlag von Paul Parey, 1912, Berlin. Ginn & Company, Boston.

THE VALUE OF THE INJECTION METHOD IN THE STUDY OF LYMPHATIC DEVELOPMENT[1]

OTTO F. KAMPMEIER

From the Laboratory of Comparative Anatomy, Princeton University

SIX FIGURES[2]

Two papers have recently been published by Professor Sabin[3] and Dr. Eliot Clark[4] in which they advance several criticisms against the theory of the direct mesenchymal origin of lymphatics, and attempt to prove that blind and discontinuous anlagen of an incipient lymphatic vessel do not exist as such, but in reality are continuous with one another. Probably their strongest contention claims that whenever a developing lymphatic duct exhibits discontinuity such a condition rests on a faulty interpretation, or is due to limitations in the study of serial sections and to the inability of the eye to discover the continuity even with the aid of high magnifications. They see in the injection method the power to demonstrate that the anlage of a lymph channel during its genesis and growth never manifests a phase of consecutive isolated segments or spaces, and they maintain that wax reconstructions which show such segments are consequently inadequate, inaccurate and fragmentary. They further contend that many of the spaces described as lymphatic anlagen by Huntington[5]

[1] Read before the American Association of Anatomists at their twenty-eighth session, December, 27, 28 and 29, 1911.

[2] Expense of illustrations borne by author.

[3] Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. Anat. Rec., vol. 5, no. 9, 1911.

[4] Eliot R. Clark: An examination of the methods used in the study of the development of the lymphatic system. Anat. Rec., vol. 5, no. 8, 1911.

[5] George S. Huntington: Ueber die Histogenese des lymphatischen Systems beim Säuger-embryo. Verhandl. d. Anat. Gesellsch., 1910. The anatomy and development of the systemic lymphatic vessels in the domestic cat. Memoirs of The Wistar Institute of Anatomy and Biology, May, 1911.

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and McClure,[6] in their studies on the development of the thoracic duct and mesenteric lymphatics in the cat, are quite probably artifacts or shrinkage cavities resulting from unequal fixation, since they occur in the center of the body where the fixing fluid penetrates last.[7]

During the course of the last two years the writer has studied the development of the thoracic duct in pig embryos and will soon publish in more complete form a paper dealing with the results of this investigation. Having observed the origin of the duct in the appearance and confluence of discontinuous mesenchymal spaces, he can entirely confirm Huntington's[8] essential conclusions in this respect. Invariably all of the pig embryos belonging to the critical period of the thoracic duct, that is, to the period of its inception and actual genesis, positively show a number of blind lymphatic anlagen in the pathway subsequently occupied by it. But the writer will not at this time enter into a detailed description of any of the specimens examined by him, except of series 23a of the Johns Hopkins University Embryological Collection. This pig embryo was injected with India ink and prepared by Professor Sabin and was sent to the Princeton Laboratory to illustrate, with the developing thoracic duct as an example, the 'centrifugal growth' theory of the origin of lymphatics. This theory holds that the entire lymphatic channel system arises from various radiation centers or lymph hearts by buds or sprouts, which by continual proliferation of their endothelial cells elongate peripherally and, by repeated branching and rebranching, invade gradually both the deeper-lying and the superficial regions of the body. "The thoracic duct," according to Professor Sabin's most recent view, "develops in part as a down growth of the jugular sac and in part, especially its dilated portion or cisterna chyli, as a direct transformation of the branches of the azygos veins."[9]

[6] Charles F. W. McClure: The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat. Verhandl. d. Anat. Gesellsch., 1910. Huntington and McClure: The development of the main lymph channels of the cat in their relation to the venous system. Am. Jour. Anat., vol. 6, 1907. Abstr. Anat. Rec., vol. 1, 1906–08.

[7] Sabin: 1911, p. 440.

[8] Huntington: 1911.

[9] Sabin: 1911, p. 424.

It will be seen, however, that one can scarcely imagine a more radical refutation of the criticisms mentioned, as well as of the view of the centrifugal budding of lymphatic ducts, than that offered by series 23a.

Before presenting the evidence, I wish to acknowledge my indebtedness to Professor Sabin for the privilege of studying the embryo in question.

Series 23a, an embryo measuring 23 mm. in length,[10] displays excellent fixation and preservation. The injection was also successfully carried out and is as perfect as a developing lymph vessel permits. The evidence for the discontinuous and direct mesenchymal origin of the thoracic duct is distinctly portrayed in the accompanying figures. Fig. 1 represents a simplified or schematic drawing based on an accurate reconstruction of the thoracic duct region, and the microphotographs (figs. 2, 3, 4 and 5) picture actual cross-sections taken at four different levels, as indicated on the diagram by the transverse lines and numbers.

The jugular lymph sac and that segment of the thoracic duct anlage connected with it were injected, and therefore they are shown in black on the drawing. Near the sac the most anterior portion of the embryonic duct is in the form of a broad and extensive plexus, a typical section of which is reproduced in fig. 2, illustrating the large size of the channels (*t.p.*) and the extravasations of the injection mass (*X*) into the surrounding mesenchyme.

To consider the right limb of the thoracic duct anlage first, the injected vessel extends unbrokenly backward and dextrad towards the right postcardinal (future azygos) vein as a slender channel (fig. 1), and terminates shortly below the level of the Cuvierian duct in a 'mossy' area produced by slight extravasations. Beyond this point we meet with the most decisive evidence in favor of the non-venous origin of the thoracic duct namely, a clear case of discontinuity, than which nothing could be more conclu-

[10] When it is considered that the processes of fixing and hardening reduce the length of pig embryos, for example, by one to one and a half millimeters, it is seen that this 23 mm. embryo (series 23a) measured before fixation, is approximately equivalent in age to 21.5 mm. embryos, the length of which was obtained after fixation.

sive. Immediately following the injected vessel is a long blind fusiform space, but in no way connected with it, as exemplified by the drawing and the microphotographs. In fig. 3 the position of the mossy extravasations from the end of the injected vessel is indicated at *X*, ventral to the broad lumen of this long independent space (*t.d.*), the section having just passed through its most anterior tip or beginning (fig. 1). That there is absolutely no open communication between these two segments of the thoracic duct anlage is strikingly confirmed by both observation and experiment. In the first place the most critical examination with the aid of high magnifications was not able to detect continuity, and secondly, not a particle of the injection mass was found to have entered the cavity of the blind space, although the pressure of the injection was sufficiently great to produce the extravasations referred to above.

The long fusiform lymphatic space of the right side is of considerable length (fig. 1), capable of being followed through thirty-seven sections (thickness of sections: 20 micra), and is variable in diameter, at times being broad, and at other times narrow and not so sharply demarcated from the intercellular lacunae of the tissue surrounding it. In form it is very irregular, and its lumen is often bridged by mesenchymal strands of greater or lesser thickness which give to it a multilocular appearance as shown in figs. 4 and 5 (*t.d.*). This condition, coupled with the fact that it is bounded by ordinary unspecialized tissue cells, supplies strong proof against the view of its venous origin. Fig. 5 also illustrates the occasional circumclusion of venules (*v.*) by this space and draws even more plainly the distinction between lymphatics and venous channels, where the latter are replete with blood and possess clearly defined boundaries as compared with the often indefinite outlines of a lymphatic anlage. Caudally the long space becomes more indistinct until it vanishes by the loss of its cavity in the confusion of the interstices of the mesenchymal reticulum, but after a number of sections it is followed by a second space, which though shorter and simpler (fig. 1) exhibits the same peculiarities of character. This again is followed by tissue, which, as yet undifferentiated, is coarsely reticulate and persistently sug-

gests the potentiality of further lymphatic anlagen. Both of the spaces described and figured are situated in the same axis of the injected channel and consequently in the axis of the ultimately complete thoracic duct.

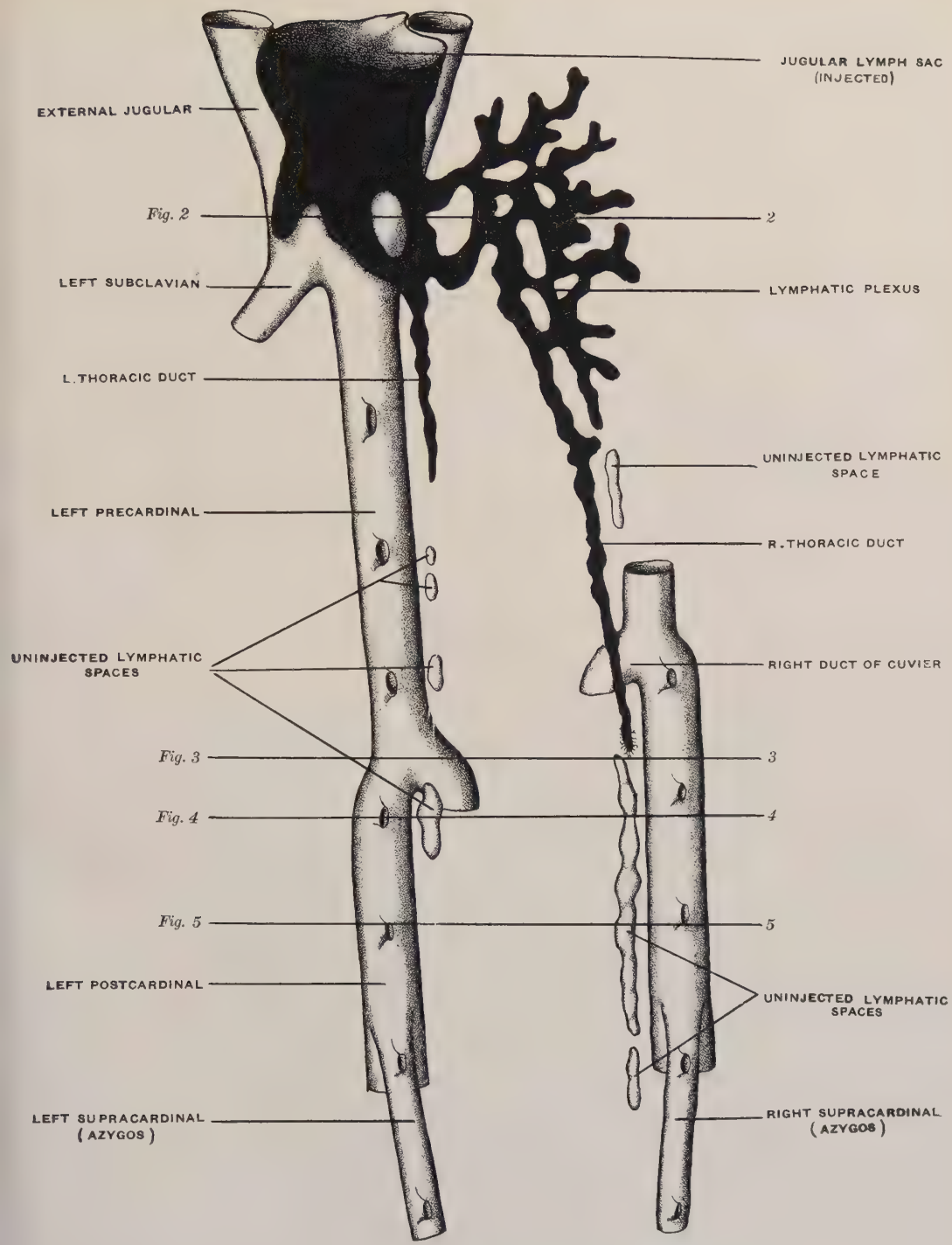
On the left side in series 23a we find the principle of development to be the same and to be expressed fully as well. The injected segment of the left thoracic duct is shorter (fig. 1) and more slender than its homologue on the right side, and often it can only be traced by a mossy path due to slight extravasations. At intervals beyond the farthest extent to which the injection mass has penetrated and located in a line destined to become the pathway of the future thoracic duct are a number of small blind mesenchymal vacuoles, the largest one extending through eight sections at the level of the Cuvierian duct. The conspicuous size of the lumen of the last space, the mesenchymal strand bisecting it, and the compactness of the neighboring tissue are clearly shown in the photograph, fig. 4 (*t.s.*).

From the observed facts it is seen that the argument for continuity in the anlage of a lymph channel and its demonstration by the method of injection do not hold, at least not in the case of the developing thoracic duct in pig embryos. Instead, the use of the injection method coupled with the study of serial sections emphasizes the discontinuities in the incipient duct. Herein lies the primary value of this technic; it defines the territory in which the fundamental period of development of such a channel has already been completed. But to arrive at a knowledge of the histogenic processes in this history it is found to be practically useless; for it is obvious that the injecting substance obscures the exact outlines of a channel, and therefore a comparison between the cells bounding the lumen and the cells of the surrounding tissue is made impossible.

On the evidence of the few microphotographs inserted here the conspicuous presence of the spaces designated by the writer as thoracic duct anlagen can not be denied. Nor can they be regarded as artifacts, for not only is their normal appearance in the figures contrary to such a view, but also the fact that they occur only in the pathway of the subsequent duct and its tribu-

taries, and are not found elsewhere in this region. Moreover, from the evidence of a large number of pig embryos it can be firmly established that these multiple spaces or anlagen appear only at a definite period in embryonic history, during the critical genetic stages of the thoracic duct, or in other words, just preceding its completion as a continuous structure. An examination of embryos of the proper consecutive ages will show plainly such anlagen beginning as minute mesenchymal vacuoles, which become progressively larger (figs. 1, 4 and 5), longer, and finally confluent with one another in a general centrifugal direction. The extent to which the injection mass will penetrate at any given moment of development therefore indicates or measures the distance in which such confluence has already occurred. Fig. 6 represents a transverse section from a later stage (26 mm. pig embryo), the thoracic duct of which has become a continuous channel throughout from the jugular lymph sac to the cisterna chyli and the mesenteric and posterior lymphatics. Taken at approximately the same level as fig. 4, this figure may be compared with it as showing the right and left limbs of the duct in the same topographical position as the spaces which have been described as its anlagen. If the discontinuous anlagen of the thoracic duct, as shown in the sections of series 23a, were to be regarded as artifacts, it appears to the writer that the same contention should apply with equal force to the continuous thoracic duct as shown in transverse section in fig. 6, especially to the right limb which is seen to be no more definite in outline or boundaries than the space illustrated in figs. 4 and 5 (*t.d.*).

Fig. 1 A simplified or schematic drawing of an accurate reconstruction (dorsal view) of the thoracic duct region in the pig embryo, series 23a (Johns Hopkins University Embryological Collection). The lymph sac and the injected portion of the thoracic duct anlage are shown in black; the uninjected lymphatic spaces are discontinuous but are located in the axes of the injected channels, and consequently in the pathway of the future complete thoracic duct. The cross lines indicate the levels at which figs. 2, 3, 4 and 5 were taken.


JUGULAR LYMPH SAC
(INJECTED)
EXTERNAL JUGULAR
Fig. 2
2
LEFT SUBCLAVIAN
LYMPHATIC PLEXUS
L. THORACIC DUCT
UNINJECTED LYMPHATIC
SPACE
LEFT PRECARDINAL
R. THORACIC DUCT
UNINJECTED LYMPHATIC
SPACES
RIGHT DUCT OF CUVIER
Fig. 3
3
Fig. 4
4
Fig. 5
5
LEFT POSTCARDINAL
UNINJECTED LYMPHATIC
SPACES
LEFT SUPRACARDINAL
(AZYGOS)
RIGHT SUPRACARDINAL
(AZYGOS)

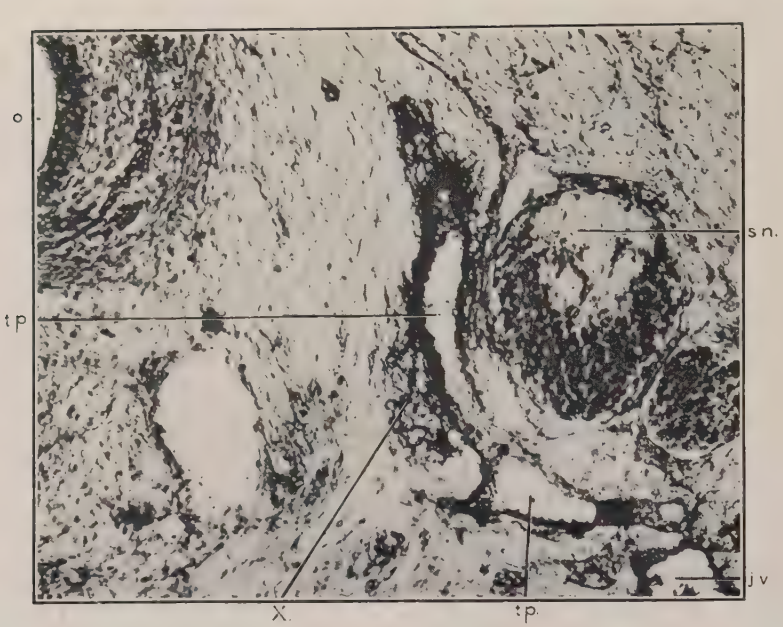


Fig. 2 Transverse section through the left lower cervical region in pig embryo 23a (J. H. U. E. C., slide 21, section 16), × 200; *t.p.*, anterior lymphatic plexus of the thoracic duct, injected; *X.*, extravasations of the injection mass into the surrounding mesenchyme; *j.v.*, internal jugular; *s.n.*, sympathetic nerve trunk; *o.*, oesophagus.

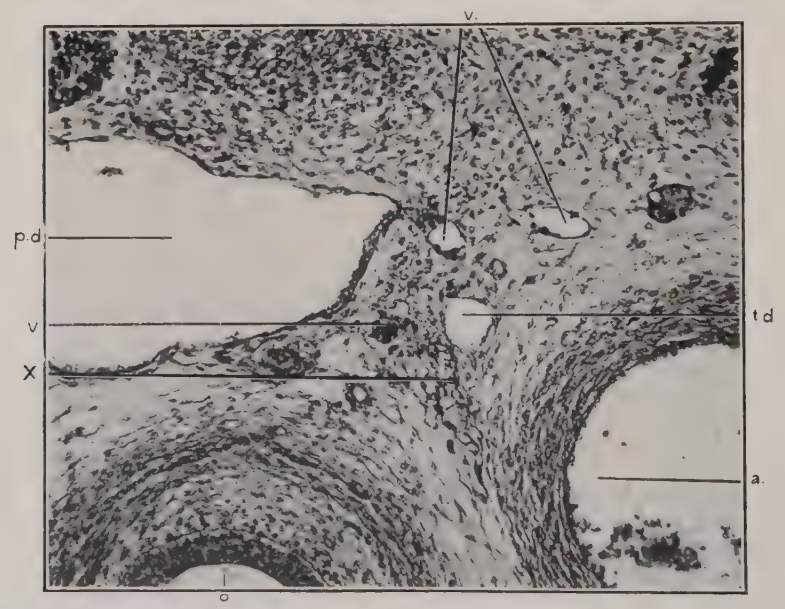


Fig. 3 Transverse section taken near the level of the right Cuvierian duct in pig embryo 23a (J. H. U. E. C., slide 26, section 10), × 200; *t.d.*, anterior tip of the long fusiform lymphatic space of the right thoracic duct line; *X.*, position of extravasated particles from the injected portion of the right thoracic duct; *v.*, venules, derivatives and tributaries of the postcardinal vein (*p.d.*); *a.*, aorta; *o.*, oesophagus. The more delicate lining of the lymphatic space as compared with that of the veins and venules can be clearly distinguished in the figure.

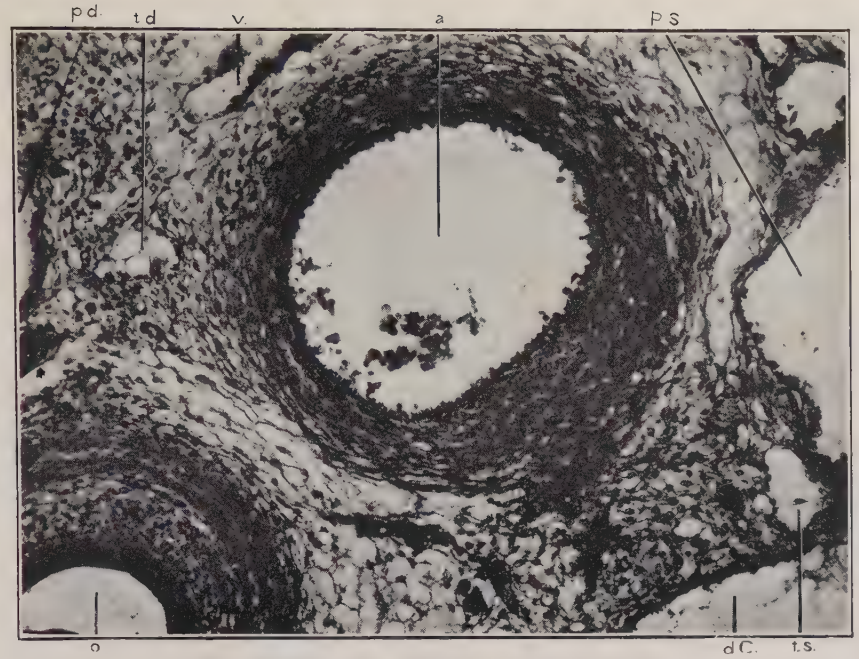


Fig. 4 Transverse section taken shortly beyond that of the preceding figure (series 23a, J. H. U. E. C., slide 26, section 12), × 200; *t.d.*, long fusiform space in the right thoracic duct line, and mesenchymal bridges traversing its lumen; *t.s.*, blind lymphatic space at the level of the left Cuvierian duct (*d.C.*), and in the path of the left thoracic duct; *v.*, venules, branches of the postcardinals; *p.d.*, *p.s.*, right and left post-cardinals; *a.*, aorta; *o.*, oesophagus.

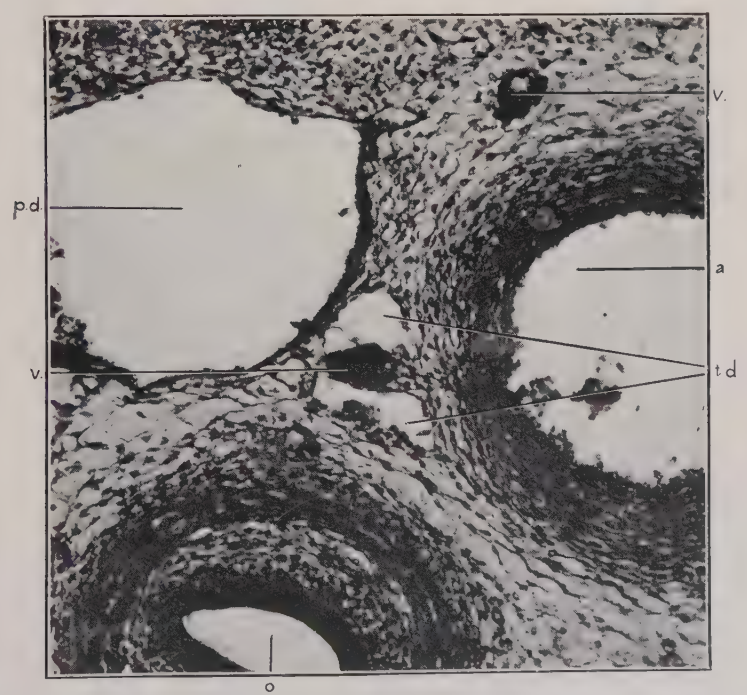


Fig. 5 Transverse section through the thoracic region in pig embryo 23a (J. H. U. E. C., slide 27, section 18), × 200; *t.d.*, long fusiform lymphatic space in the right thoracic duct line, and surrounding a venule (*v.*), or tributary of the right post-cardinal vein (*p.d.*,); *a.*, aorta; *o.*, oesophagus.

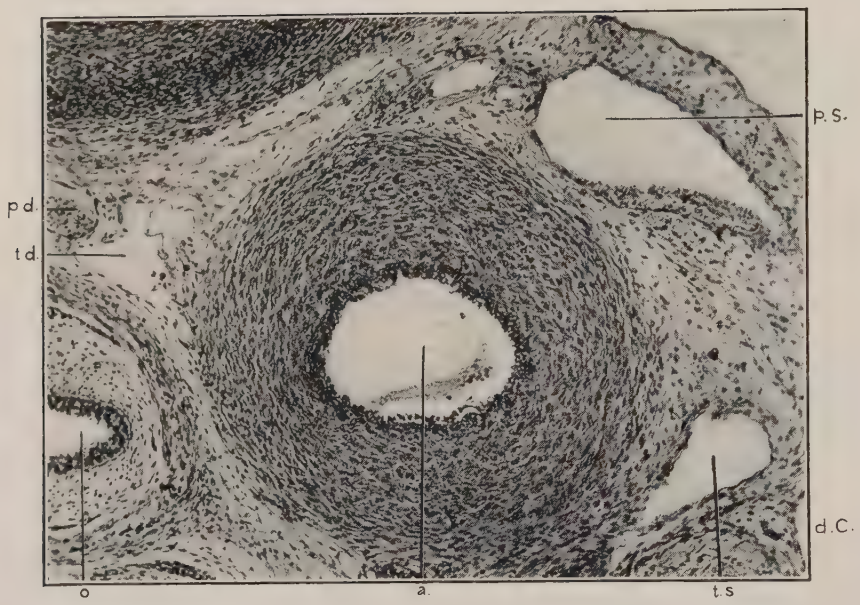


Fig. 6 Transverse section taken near the level of the left Cuvierian duct in a 26 mm. pig embryo (series 69, Princeton Embryological Collection, slide 42, section 9), × 150; *t.d.*, *t.s.*, right and left thoracic ducts; *p.d.*, *p.s.*, right and left postcardinals; *d.C.*, left Cuvierian duct; *a.*, aorta; *o.*, oesophagus.

A FEW REMARKS RELATIVE TO MR. KAMPMEIER'S[1] PAPER ON 'THE VALUE OF THE INJECTION METHOD IN THE STUDY OF LYMPHATIC DEVELOPMENT'

CHARLES F. W. McCLURE

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At the annual meeting of the American Association of Anatomists, held at Ithaca in December, 1910, Professor Sabin read a paper on the development of the thoracic duct in the pig in which she maintained that this duct grew from a lymph sac, of venous origin, in a continuous manner and in a centrifugal direction and was not formed as the result of a fusion between multiple and discontinuous anlages. This particular paper, as far as I know, has not yet appeared in print.

Since a fundamental principle was involved which concerned the development of the main lymphatic channels in general and a view was expressed diametrically opposed to the one advanced and maintained by Huntington and the writer, I asked Professor Sabin in open meeting if she would loan me a pig embryo injected and sectioned by herself which would represent a critical stage in the development of the thoracic duct at a time prior to its establishment as a continuous channel throughout its entire length. This she generously consented to do and sent me soon after the meeting the beautifully injected and sectioned series of the 23 mm. pig embryo (Johns Hopkins Embryological Collection, series 23a) which Mr. Kampmeier has already described in a separate article and which appears in the same number of The Anatomical Record as the present paper.

This embryo was given by me to Mr. Kampmeier for study, for the reason that he had already nearly completed an investi-

[1] Kampmeier, O. F.: The value of the injection method in the study of lymphatic development. Anat. Rec., vol. 6, no. 6, 1912.

gation dealing with the development of the thoracic duct in the pig.

I wish to thank Professor Sabin again for her courtesy as well as for the privilege she gave me, both verbally and in writing, of publishing the conditions which Mr. Kampmeier has found and which I predicted at the meeting would be found in such an embryo.

A few months after the meeting, in the spring of 1911, I sent to Professor Sabin two accurately and carefully drawn reproductions (dorsal and ventral views) of the reconstruction which Mr. Kampmeier had made of the veins, venolymphatics, lymphatics, arteries and nerves in the thoracic region of the embryo in question, as well as the photomicrographs of the uninjected lymph spaces which are reproduced in his paper. Apparently the evidence which I sent to the Johns Hopkins Anatomical Laboratory did not meet with approval by those interested in the problem of lymphatic development at that Institution for, after receiving this evidence, two papers were published by Professor Sabin[2] and Dr. Clark,[3] respectively, in which no mention of this evidence is given and in which they attempt to clear up the problem of the lymphatics by a critical review of the methods employed by various investigators.

Regarding methods in general Professor Sabin ('11, p. 417) states that:

> A review of the recent work on the development of the lymphatic system shows that there are marked differences of opinion on fundamental points and a study of these differences will demonstrate that they center around the question of methods. We differ because our methods differ and therefore it is essential to submit the methods to comparative tests and to understand the limits of error of each one.

If we differ because our methods differ and it is essential to submit the methods to comparative tests and to understand the limits of error of each one, it seems strange to me that Professor Sabin

[2] Sabin, F. R.: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. Anat. Rec., vol. 5, no. 9, 1911.

[3] Clark, E. R.: An examination of the methods used in the study of the development of the lymphatic system. Anat. Rec., vol. 5, no. 8, 1911.

has not put to a comparative test the pig embryo of the Johns Hopkins Embryological Collection (series 23a), which was injected and sectioned by herself and which shows in a most unmistakable manner not only the limits of error entailed by the use of the injection method but, equally well, the reason why we differ on fundamental points. It appears to me that this embryo would have served as an excellent example in proof of the utter inadequacy of the injection method as a means of determining the principle of lymphatic development. Professor Sabin could scarcely have sent me an example more conclusive of the fact that the thoracic duct is formed through the fusion of a number of discontinuous anlages and not as an outgowth from a lymph sac. As a matter of fact, however, any pig embryo of about the same age as that of the Johns Hopkins series (23a) would present, approximately, the same fundamental conditions, namely, a right and left thoracic duct, ending blindly in the thoracic region which can be injected from the jugular lymph sac and, caudal to these ducts and lying in the same axes, a series of isolated and independent lymph spaces formed in the mesenchyme, which later become joined in a centripetal direction to the injectible portion of the thoracic ducts, as a means of increasing the extent of their channel system (see Kampmeier's fig. 1).[4]

It has therefore seemed essential for Mr. Kampmeier to make this comparative test because Professor Sabin states ('11, p. 433):

> That the injection method used alone is unreliable is barely worth an answer, for no competent investigator uses it alone but always in comparison with uninjected specimens.

Professor Sabin ('11) also states on page 432 that:

> Huntington and McClure have criticized the injection method by saying that it fails to demonstrate all the lymphatics. They say, 'Injection will, if successful, only demonstrate channels or spaces actually continuous with each other at the time of the injection, but will completely fail in revealing vascular spaces as yet independent of those injected, although subsequently a connection between the two may be established' ('10, p. 180).[5]

[4] Loc. cit., p. 229.

[5] Huntington and McClure.: The anatomy and development of the jugular lymph sacs in the domestic cat (Felis domestica). Amer. Jour. Anat., vol. 10, no. 2, 1910.

Nothing could prove more conclusively the truth of our statement than the study of series 23a of the Johns Hopkins Embryological Collection.

Professor Sabin ('08, p. 48)[6] further states that:

> Everyone who has worked on the lymphatic system in early stages is aware of the extreme difficulty in identifying lymphatics. There are so many structures with which they may be confused in sections that it seems as if almost the only clue with which to meet the difficulty is to start with the finished lymphatic system and trace it back to the younger stages.

This statement is quite correct. The ability to recognize isolated and independent mesenchymal lymphatic anlages in the mammalian embryo is only acquired after a considerable and careful study of many series of the proper stages. That Professor Sabin and Dr. Clark have not as yet acquired the facility of correctly diagnosing uninjected lymphatic anlages in sections is shown by their failure to observe the thoracic duct anlages present in the pig embryo of their own collection (series 23a).

If the thoracic duct is formed through the fusion of independent and isolated anlages, as proved by the embryo under discussion, it is improbable that the same principle is not involved in the development of other systemic lymph channels of the embryo.

We now turn our attention to Professor Sabin's latest opinion concerning the development of the thoracic duct:

> The thoracic duct develops in part as a down growth of the jugular sac and in part, especially its dilated portion or cisterna chyli, as a direct transformation of the branches of the azygos veins. These observations must be considered in the light of the tests on limitations of methods to be brought out later (Sabin, '11, p. 424).

Professor Sabin ('01–2)[7] at first held that the anlages of the thoracic duct started as two blind ducts which budded off directly from the veins in the neck and inguinal regions and finally met

[6] Sabin, F. R.: Further evidence on the origin of the lymphatic endothelium from the endothelium of the blood vascular system. Anat. Rec., vol. 2, nos. 1 and 2, 1908.

[7] Sabin, F. R.: On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. Amer. Jour. Anat., vol. 1, no. 3, 1902.

somewhere in the thorax where they joined to form a continuous channel. Now in her most recent statement on this subject only the anterior portion of the duct does the budding, not directly from the veins, but from a lymph sac formed through the fusion of a detached plexus of veins, while the rest of the duct, especially the cisterna chyli, is formed in part as a direct transformation of the branches of the azygos veins.

One reason for this change of opinion may be due to the fact that:

> The thoracic duct has proved to be the most difficult part of the lymphatic system to work out for this reason, we have not yet found a way to inject it in early stages and uninjected sections are not adequate (Sabin, 1909, p. 77).[8]

I hope that a careful revision of her series 23a, in the light of the interpretation given by Mr. Kampmeier, will convince Professor Sabin that uninjected sections are quite as 'adequate' as injected ones if studied carefully and with understanding.

In view of her recent statement concerning the development of the thoracic duct, of the impossibility of injecting a detached plexus of veins from the systemic veins and of the conditions present in the injected pig embryo which Professor Sabin sent to the Princeton Laboratory, the following quotation from her most recent paper is also interesting (Sabin, '11, p. 424).

> I maintain and can prove by injections that connections can be demonstrated between apparently isolated lymphatics which can not be found in sections of uninjected specimens. Therefore in a new territory one can not be certain of the extent of the lymphatics without injections.

If the reader will turn to fig. 1 of Mr. Kampmeier's[9] paper he will see that what Huntington and I regard as 'isolated lymphatics' in an uninjected specimen are also 'isolated lymphatics' in an injected one.

Both Professor Sabin and Dr. Clark have made interesting 'tests' in which they attempt to prove that the apparently isolated

[8] Sabin, F. R.: The lymphatic system in human embryos. Amer. Jour. Anat., vol. 9, no. 1, 1909.

[9] Loc. cit., p. 229.

endothelial-lined spaces found in sections of the embryo are in reality connected with one another in the living form.

Professor Sabin sectioned a 27 mm. pig embryo in which the right jugular lymph sac and the peripheral vessels leading to it had been injected. A reconstruction was then made of the vessels in the corresponding areas on the injected and uninjected sides. Speaking of the reconstruction of the injected area she states ('11, p. 429) that:

> In fig. 7 is shown the reconstruction of the injected plexus in which the vessels are shown in solid black and the extravasations in dotted areas. The injection was almost complete, there being only one endothelial-lined vessel not containing ink but it was adjacent to an extravasated area. A few of the vessels connected with the main plexus through extravasations so dense that the endothelial wall could not be traced. The fundamental point is well brought out that an elaborate plexus which has been injected can be reconstructed almost completely, leaving very few isolated islands. On the other hand, fig. 8 shows a reconstruction of the same area on the uninjected side, the drawing of every section having been tested in exactly the same way with the 4 mm. lens and all endothelial sprouts of which I could be sure added before the reconstruction was made. It must be emphasized that the drawings include only endothelial-lined spaces and that these endothelial-lined spaces corresponded with vessels on the opposite side which are known to be lymphatics through their connection with the lymph heart. The figure shows many of the isolated lymphatics, or Mayer-Lewis anlages. These are true lymphatics. This test demonstrates the point which I brought out in 1908, pp. 53-54, that one can inject more of the true lymphatic plexus than one can reconstruct and that an uninjected lymphatic capillary plexus can not be fully reconstructed but rather breaks into the Mayer-Lewis anlages.

Disregarding the possibility that a complete plexus had not yet been established on the uninjected side, the point Professor Sabin wished to establish by her test is that every single one of the isolated endothelial-lined islands or 'Mayer-Lewis anlages' which she found on the uninjected side were really connected with one another and with the jugular lymph sac. If this is not the case what was the object of the test? Suppose the injected side of the 27 mm. pig embryo had, prior to the injection, contained isolated and hence uninjectible lymph spaces such as are present in series 23a of the Johns Hopkins Collection, would these spaces have been recorded as endothelial-lined vessels 'not containing

ink' but 'adjacent to an extravasated area' or 'vessels connected with the main plexus through extravasations so dense that the endothelial wall could not be traced?' To the unprejudiced observer the element of uncertainty in the injection method lies in the extravasations of the injected ink and to any one familiar with the delicate limiting tissues of developing lymphatic anlages the occurrence of these extravasations is not a matter of surprise.

Dr. Clark attempted to reconstruct from sections the same plexus which he had already studied in the tail of a living tadpole (Hyla pickeringii) and thus eliminates all possibility that this plexus was not originally complete. Concerning his conclusions, Dr. Clark ('11, p. 407) states as follows:

> The complete lymphatic system as shown in fig. 5 could not be reconstructed under the most favorable conditions. Thus a careful testing of the method of reconstruction from serial sections proves that this method is unreliable for the study of the development of the lymphatic system, for since it is impossible to reconstruct known vessels the method cannot demonstrate the extent of unknown vessels.

If the object of Dr. Clark's 'test' was to prove that the tail of the tadpole he examined was so badly shrunken that a capillary plexus could not be followed in sections, he succeeded most admirably. If, on the other hand, the object of his 'test' was to establish the general principle that the hitherto described independent and isolated anlages of the lymphatic system do not exist as such but are merely segments of a shrunken capillary plexus, that is another question. I would then refer him to the injected pig embryo of the Johns Hopkins Embryological Collection (series 23a) in which he can see at a glance that, as far as establishing a principle is concerned, his 'test' has proved a waste of time and energy when applied to the development of the thoracic duct.

Some may claim that the uninjected lymph spaces in series 23a, as figured by Mr. Kampmeier, have not been reached by the injection mass on account of the incompleteness of the injection. Dr. Clark, however, dispels this idea as far as this particular embryo (series 23a) is concerned when he says ('11, p. 412) that:

Too little pressure fails to fill all the capillaries to their ends. With too great pressure there is produced a mossy appearance around the capillary, as has been pointed out by Hoyer, due evidently to forcing the injection mass through the lymphatic wall.

One might ask Dr. Clark here whether in the latter case the injection mass is forced 'through the lymphatic wall' as the result of a break in the same or whether it passes through an intact membrane? I ask this question chiefly for the reason that a 'mossy' appearance is evident at the end of the right thoracic duct and, in fact, is apparent here and there along the entire length of both thoracic ducts in the Johns Hopkins series 23a.

Dr. Clark fully agrees with Professor Sabin when he says:

In general it may be stated that when used with care the injection method may be relied upon to demonstrate the full extent of the lymphatic system at any given stage. In comparison with the method of reconstruction from serial sections the injection method is surely superior ('11, p. 413).

Dr. Clark ('11, p. 413) also states that:

Though the injection method is much more reliable than the study of cross sections to determine the extent of the lymphatic system at any stage, many questions as to the exact way in which growth takes place cannot be determined by it.

Until this last statement had been made one might have thought that the method of injection had no limitations. The truth of Dr. Clark's qualification, however, is amply sustained by a critical study of the Johns Hopkins specimen, series 23a.

We must now pass to another interesting topic, the question of the degeneration of capillaries.

After stating that she can prove by injections that connections can be demonstrated between apparently isolated endothelial-lined spaces which cannot be found in uninjected specimens, Professor Sabin now confronts us with the statement that a few detached vessels, either lymphatics or blood vessels, may also be present.

I am glad to see, however, that Professor Sabin and Dr. Clark finally recognize the existence of certain detached endothelial-lined vessels in the embryo, to which Dr. Huntington and I have

repeatedly called their attention, because such vessels form in the mammalian embryo important static lines along which a large proportion of the main lymphatic channels develop, especially the thoracic duct and mesenteric channels, by confluence of perivenous or, as we have briefly termed them, 'extraintimal' mesenchymal spaces.

Professor Sabin speaks of these detached vessels as follows ('11, p. 431):

> The question of the degeneration of capillaries should also be considered in connection with the Mayer-Lewis anlages. It was first observed by Ranvier ('95–'96) that in the mesentery of the pig there were isolated endothelial-lined spaces separate from the lymphatic plexus which he interpreted as degenerating lymphatics. Dr. Clark has observed such a separation of islands from the rest of the plexus and their subsequent disappearance but it is not a common occurrence. Vessels usually retract instead of becoming cut off in a growing zone but their separation does sometimes occur. From these studies of Dr. Clark and myself, it is a legitimate conclusion, that the Mayer-Lewis anlages, which are the isolated endothelial-lined islands in sections of vascular and lymphatic areas, are all true vessels, either blood vessels or lymphatics whose endothelium is derived from previous endothelium. With the exception of a few degenerating vessels, the Mayer-Lewis anlages connect in the living specimen, and can be injected. They stand in the strongest contrast to the mesenchyme spaces on account of their endothelial lining. Thus the Mayer-Lewis anlages we have been studying are lymphatics.

What interests me in particular is to find out how Professor Sabin was able to distinguish the detached and isolated endothelial-lined veins or lymphatics from the other endothelial-lined Mayer-Lewis anlages which are all true vessels. In view of her 'test' she certainly could not have determined their presence in sections of an uninjected pig embryo, because she claims that the endothelial-lined islands which appear to be isolated in sections are in reality true vessels and can be injected. If such is actually the case and she can prove it by injections, I fail to see what endothelial-lined spaces would then be left over after injection which she could regard as detached islands. In the case of one of her 'tests,' where an endothelial-lined island was separated from the injected plexus by an extravasation, would this island be regarded by her as a detached vessel? It seems to me that her 'test' attempted to prove that it should not be so considered (Sabin,

'11, p. 429). If this island is not detached in this particular injected pig embryo, on what basis would she diagnose detached vessels in others?

Has it actually been possible for Professor Sabin to inject isolated endothelial-lined islands which do not connect with the vascular system, by injecting them through functional venous or lymphatic channels, or, has she been able to make an independent injection of each one of these islands? If the latter, how did she know to begin with that the island in question which she wished to inject was detached? If, as she claims in Keibel and Mall's Handbuch der Entwicklungsgeschichte des Menschen (Band 2, S. 692), that in 1908 she was able to confirm Lewis' conclusion on pig embryos, by means of the injection method, that the jugular lymph sacs were formed through a fusion of detached veins, she must have been able to perform both of these feats.

What is really meant by a 'Mayer-Lewis anlage?' An anlage must be the anlage of something. As far as I can determine Professor Sabin believes in the existence of four varieties of 'Mayer-Lewis anlages' which, as they appear in sections, are as follows: (1) Sections of functional lymphatic capillaries; (2) sections of functional venous capillaries; (3) sections of detached lymphatics which degenerate; and (4) sections of detached veins which degenerate.

If, as Professor Sabin states ('11, p. 432), "the Mayer-Lewis anlages, which are the isolated endothelial-lined islands in sections of vascular and lymphatic areas, are all true vessels, either blood vessels or lymphatics whose endothelium is derived from previous endothelium" and "with the exception of a few degenerating vessels, the Mayer-Lewis anlages connect in the living specimen, and can be injected," it is difficult for me to understand where the detached veins come in which do not degenerate and by what 'tests' or by what other methods of investigation she was able to determine their presence. Because she now claims that the thoracic duct develops in part, especially its dilated portion, the cisterna chyli, as a direct transformation of the branches of the azygos veins (Sabin, '11, p. 424).

Has it ever occurred to Professor Sabin that her line of reasoning has led us to infer that she had been injecting detached vessels which degenerate but which at the same time enter into the formation of the jugular sacs and the thoracic duct? Because, if transformed branches of the azygos veins should develop into the cisterna chyli or any other part of the thoracic duct, most of us would agree that these veins must necessarily become detached from the main venous channels and that they can not undergo degeneration if they aid in forming lymphatic structures. We have been informed, however, that the detached vessels, both lymphatics and veins, undergo degeneration. If some of the detached veins do not degenerate, possibly we have room here for what Professor Sabin might be pleased to call a fifth variety of a 'Mayer-Lewis anlage.' But this is not all. In view of the 'test' she has made, how was she able by means of sections, either of an injected or uninjected specimen, to come to the following conclusions?

> It is not possible to set limits to the transformation of veins into lymphatics making the cisterna chyli and thoracic duct, for by comparing the two specimens measuring 23 mm. it can be seen that vessels which are clearly branches of the azygos veins in one specimen do not seem to connect with the vein in the other (Sabin, '11, p. 423).

By whatever method it may have been determined by her, Huntington, Kampmeier and the writer can verify Professor Sabin's statement that the vessels in question do become detached from the main venous channels. So far Professor Sabin's observations are correct. Curiously enough, however, Professor Sabin has failed to recognize, in this particular instance and in accordance with her expressed views, that these detached vessels actually do degenerate and that the thoracic duct is not formed through a fusion of vessels detached from the azygos veins, but through a fusion of mesenchymal spaces which merely utilize the static line vacated by the detached vessels after their degeneration has been accomplished. These mesenchymal lymph spaces (*td*, *ts*) may be seen in Mr. Kampmeier's[10] figs. 3 to 5, inclusive, which are photomicrographs of sections of a 23 mm. pig embryo belong-

[10] Loc. cit., pp. 230 and 231.

ing to the Johns Hopkins Collection (series 23a). One of the tributaries (*v*) which has not yet been detached from the azygos vein, may be seen in fig. 5 partially surrounded by the independently formed mesenchymal lymph spaces (*td*). At a slightly later period of development, however, this tributary would become detached from the azygos vein and degenerate and, together with the surrounding mesenchymal lymph spaces, it would then present a picture which Huntington and I have described as the 'extraintimal' replacement of a degenerating vessel by a lymphatic. It is evident, and so appears clearly in our publications, that the process of 'extraintimal' replacement is only an adaptation of the basic principle of lymphatic genesis, through the fusion of independent mesenchymal spaces, to the local conditions which prevail in certain districts of the mammalian embryo.

Although Professor Sabin may claim that her position has always been a consistent one; on the ground that from the beginning she has maintained that the endothelium of the lymphatics is derived from the veins, one might ask what conclusive proof, not inference, she has thus far given us that such is actually the case.

After a critical analysis of her writings, it appears to me that the present illogical position in which Professor Sabin has placed herself, with resulting confusion to all of us, is largely due to the circumstance that it is often disagreeable to acknowledge, in an emphatic and unmistakable manner, any change of opinion or modification of one's views.

While reading the publications of Professor Sabin and Dr. Clark it has occurred to me that there may be nine possible ways of interpreting the origin and significance of the isolated and independent spaces in series 23a of the Johns Hopkins Embryological Collection, to which attention has already been called by Mr. Kampmeier. It has also occurred to me that the correct answers to these questions, backed by evidence, might not only relieve the minds of a long suffering public, but might also clear the way for a complete understanding of the fundamental principles involved in the development of the main lymphatic channels in the mammalian embryo.

These questions are as follows: (1) Are the spaces which are labelled *t.d.* and *t.s.* in Mr. Kampmeier's figs. 3 to 5, inclusive, cross sections of a true lymphatic which is still in connection with one of the primary lymph sacs ('Mayer-Lewis anlage,' Variety 1)? (2) Are these spaces cross sections of a true vein still in connection with the main venous channels ('Mayer-Lewis anlage,' Variety 2)? (3) Are these spaces cross sections of detached lymphatics which degenerate ('Mayer-Lewis anlage,' Variety 3)? (4) Are these spaces cross sections of detached veins which degenerate ('Mayer-Lewis anlage,' Variety 4)? (5) Are these spaces cross sections of detached veins which, in Lewis' sense, may aid in forming the thoracic duct? (6) Are these spaces rents or tears in the mesenchyme which do not appear uniformly in the ontogenetic development of the thoracic duct and are therefore artifacts? (7) Are these spaces the 'true rounded spaces in the mesenchyme which undoubtedly contain lymph but which are not a part of the lymphatic system' which were described by Professor Sabin in 1908 (p. 51)? (8) Are these spaces perineural spaces? (9) Are these spaces real lymph spaces which develop in the mesenchyme, independently of the intima of the veins or that of the injected portion of the thoracic duct or of any other lymph channel, which would subsequently unite in a centripetal manner with the injected portion of the thoracic duct, as a means of completing its channel system?

Finally, as a sort of an appendage to the above questions, I might ask the following one: In case it should be finally acknowledged by all that the spaces labelled *td* and *ts* in Mr. Kampmeier's[11] figs. 3 to 5, inclusive, actually do aid in completing the channel system of the injected portion of the thoracic duct in series 23a, would they be added to it in a centrifugal or in a centripetal direction?

If any misunderstanding has existed in the past regarding the presence and general character of the mesenchymal lymph spaces which Huntington and I believe enter into the formation of the lymph channels there need be none in the future, for the reason

[11] Loc. cit., pp. 230 and 231.

that these spaces have been clearly and decisively pictured by Mr. Kampmeier (figs. 1 to 5, inclusive) in his description of the injected and sectioned pig embryo which Professor Sabin kindly loaned to the Princeton Laboratory.

It appears to me that Dr. Clark states very fairly Professor Sabin's position when he says ('11, p. 412):

> In studying the lymphatics in the skin of embryo pigs with the aid of injections, Miss Sabin found that, starting from the region of each of the four primitive sacs, there is a gradually increasing zone of injectible lymphatics which eventually covers the entire body. She was unable to find by injection any remaining isolated lymphatics, and was naturally led to the conclusion that the growth of the system was a centrifugal one taking place by the sprouting of preëxisting endothelium.

The fact that "she was unable to find by injection any remaining isolated lymphatics, and was naturally led to the conclusion that the growth of the system was a centrifugal one taking place by the sprouting of preëxisting endothelium," is the very reason why Huntington and I have contended from the beginning that her theory is based on inference and not on facts. If this is not the case, why should it be left to Mr. Kampmeier to point out to her in her own material the presence of the 'remaining isolated lymphatics' which lie beyond the field of an injected vessel?

It is easy to comprehend how, in the case of her specimen (series 23a), the appearance of a centrifugal growth might be simulated by the thoracic duct, by reason of a progressive centripetal addition of lymph spaces to the injectible portion of the duct. It is evident, however, that this method of growth or development, as she may wish to call it, differs fundamentally from the view maintained by her that the growth of the lymphatic system is a centrifugal one taking place by the sprouting of preexisting endothelium.

GENERAL OBSERVATIONS ON EARLY SUPERFICIAL LYMPHATICS IN LIVING CHICK EMBRYOS

PRELIMINARY NOTE

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Last fall an attempt was made to study the development of the superficial lymphatics of the chick by injections with India ink. In chicks of seven days and over it was very easy to study the character and extent of the lymphatics by this method, but in younger embryos I found it increasingly difficult to obtain satisfactory lymphatic injections, since in almost every case the superficial blood capillaries were injected at the same time. However, it was occasionally possible to inject an irregular plexus over the pelvis, and from it a deeper plexus connected with the intersegmental coccygeal veins, in the exact region occupied by the functioning posterior lymph heart of older embryos.

A living chick of five days and twenty hours was then examined under the high power binocular microscope, in bright sunlight. Care was taken in opening the shell not to injure the embryo, and the yolk sac was kept intact. Observation of the region in the tail, later occupied by the posterior lymph heart, revealed the presence of two distinct sets of vessels. The first of these was undoubtedly composed of blood capillaries. With brilliant illumination this richly anastamosing plexus could be plainly seen and the direction of circulation in the individual capillaries made out.

But in the living chick another distinct blood-filled plexus was visible. For the most part it appeared to be deeper than the superficial blood capillary plexus and nearer the coccygeal veins. This second plexus was found to be easily distinguishable from the blood capillaries in four ways: (1) the vessels composing it are in general larger than the blood capillaries,

(2) their pattern is different, (3) the blood contained in them is of a darker red color, and (4) no circulation can be observed in them. The last feature is the most striking, for in contrast to the rapid motion of the blood corpuscles in all of the capillaries of the first plexus, the blood of this second plexus remains stagnant. This plexus of vessels with stagnant blood was found to be constant in chicks of this and somewhat later stages.

Next, various tests were made to determine further the character of this plexus filled with stagnant blood, and its relation to the blood vascular system. They are as follows:

1. In a living chick a few granules of ink were injected into several of the circulating blood capillaries. The granules were seen to pass through the capillaries and venules into the main intersegmental veins, but in no case did they enter this stagnant plexus.

2. The plexus filled with stagnant blood was injected. After the discovery that at this early stage the lymphatics could be distinguished from the blood vessels in the living chick, much of the difficulty of the former injections could be avoided. A very small glass canula is used (from 12 to 15 micra in diameter at the tip) and with the aid of the high power binocular microscope, it is not difficult to insert the needle directly into a selected lymphatic capillary. Since these vessels are filled with blood, it is easy to overdistend them, but if a gentle pressure is used it is possible to obtain injections with no extravasation. When this method was substituted for the former one of plunging the needle blindly into the tissue, the neighboring blood capillaries were never injected simultaneously with the lymphatics. In all cases they remained undisturbed.

Wherever the injection of the lymphatics is pushed far enough the ink runs back from the surface vessels, into the deeper plexus, and from it into the five intersegmental coccygeal veins. At no other point does the ink ever enter the venous system from a direct injection of this early posterior plexus filled with stagnant blood.

3. The superficial blood capillaries of this region were then injected. In no case did the ink enter the stagnant plexus,

which still remained filled with blood and quite independent of the injected blood capillaries:

4. Complete blood vascular injections were obtained through one of the large vessels of the yolk sac or allantois. Here too the blood-filled lymphatic plexus remained uninjected with ink.

5. Double injections were also obtained. In one six-day chick, I injected the blood-filled lymph heart plexus with ink, stopping the injection just as it entered the coccygeal veins. The surface blood capillaries were then filled with Berlin blue. On the other (left) side of the same specimen, the superficial blood capillaries were first filled with the Berlin blue, leaving the blood in the lymph heart plexus. The main sub-cardinal vein was then injected with India ink. Its branches, the coccygeal veins, soon filled and, since the Berlin blue previously injected blocked off the blood capillary branches, the ink was forced into the blood-filled lymphatic plexus. In both cases the injections showed strikingly the lymphatic connections with the intersegmental veins and showed conclusively that there is no other communication with the blood vascular system in this posterior region.

6. Injections of the blood-filled lymphatics with silver nitrate were also obtained which showed that these early vessels possess definite endothelial markings.

7. When the large arteries and veins of the yolk sac and allantois were opened and the embryo was allowed to bleed freely, the blood could be seen to fade out of the superficial blood capillaries in which the circulation had previously been observed. However the blood did not fade from the lymphatic plexus, which remained undisturbed by the bleeding and still filled with the dark red stagnant blood.

To summarize the results of these observations:

Early lymphatics can be seen in the living chick and can be distinguished from the blood capillaries of the same region by several criteria, the most noticeable of which is that they are filled with stagnant blood.

In and near the region later occupied by the posterior lymph heart these blood-filled lymphatics are seen to form a plexus,

whose connections with five of the intersegmental coccygeal veins can readily be demonstrated by injection.

Various injection tests also show that this early lymphatic plexus is independent of the surrounding blood capillaries, for each of the two sets of vessels can be injected separately without disturbing the other.

The fact that the blood contained in this early plexus is stagnant—that is, backs up into it from the vein—shows that the early lymphatics pass through a distinct non-functioning stage.

In studying the development of the jugular lymph sac, F. T. Lewis, and later other investigators, have shown the presence of a capillary plexus preceding the formation of a definite sac. In most cases they have pictured this plexus as a group of isolated channels and vessels known as veno-lymphatics and have interpreted them as a transformed blood-capillary plexus. It has been inferred that this stage was preceded by one in which the vessels, which later are going to form lymphatics, are an integral functioning portion of the blood vascular system. In an article in this number of The Record on injection and reconstruction of the jugular lymph sac, I have shown that the appearance of isolation of parts of this early plexus, shown in reconstruction, is attributable to the limitations of the method. In the case of the posterior lymph heart, observation of the living chick shows that the early plexus is not composed of isolated vessels but is a definite continuous plexus connected with the intersegmental coccygeal veins. And the fact that the plexus is filled with stagnant blood and is therefore non-functioning, together with the fact of its being entirely separate from the surrounding blood capillaries, prevent me from regarding it as a true blood-vessel plexus. It is therefore proper to consider this plexus of vessels filled with stagnant blood as a non-functioning lymphatic plexus.

Having established the fact that the early lymphatics are filled with stagnant blood and can therefore be seen in living chick embryos, we have an entirely new method for the study of their development. In an accompanying article is given a preliminary report of the results of such a study of the very earliest lymphatics. In a later article will be published the results of studies

on later stages. In brief, it has been found that a non-functioning plexus, packed with blood and similar to the posterior lymphatic plexus, develops in the lower cervical region and under the shoulder in connection with the anterior and posterior cardinal veins near their junction to form the duct of Cuvier. The non-functioning period, during which the lymphatics are filled with blood, lasts for from twenty-four to thirty hours. During this time a rapid extension of the two plexuses takes place which may be observed in living embryos.

OBSERVATIONS ON THE DEVELOPMENT OF THE EARLIEST LYMPHATICS IN THE REGION OF THE POSTERIOR LYMPH HEART IN LIVING CHICK EMBRYOS

PRELIMINARY NOTE

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The discovery made by one of the authors that, in the chick' the earliest lymphatics are non-functioning, that they are usually packed full of stagnant blood and that they can therefore be seen in the living chick and distinguished from the blood-vessels with their circulating blood, suggested the possibility of actually watching the development of the very earliest lymphatics. The region of the posterior lymph heart, where, as Sala and Mierzejewski have shown, lymphatics develop in connection with blood vessels which are comparatively superficial, offered itself as the most favorable place for such a study.

The problem before us, then, was to keep the chick alive and under continuous observation for a sufficient length of time; to make careful records of the blood vessels during the period preceding the development of the lymphatics; to watch for the earliest appearance of the lymphatics, noting their relation to and behavior toward the blood vessels; to find out, if possible, the mode of extension, character and behavior of the earliest lymphatics; and to make tests by various injections and by the study of cross-sections in order to supplement and control the study of the living structures.

This program was carried out with the following results:

By using a warm chamber it was possible to keep chicks alive and under observation for from five to seven hours. This proved to be long enough for our purposes.

The lymphatics in the posterior lymph heart region first appear in chicks of approximately five days of incubation, measuring immediately after being placed in the fixing fluid, between 12 and 13 mm. greatest length; though we have found considerable variation in both age and length. The embryo is at approximately the stage represented in fig. 29 of Keibel and Abraham's 'Normentafeln.'

The first evidence of lymphatics in the tail region of living chicks is the appearance of a number of separate knobs, filled with stagnant blood, a little darker in color than the circulating blood, just lateral to several of the most anterior of the dorsal intersegmental coccygeal veins. The connections with the veins cannot be seen, since the knobs lie between them and the observer, but ink injected into the knobs can be seen to pass directly into the main intersegmental veins.[1] Between the separate knobs no anastomoses can be seen, nor can any be discovered by injection.

Soon after these knobs appear (in about fifty-five minutes), similar ones develop about them which have fine connections with them, thus forming a small cluster. The new ones are located partly on either side of the first and partly superficial to them. Their injection now shows discreet tiny clusters, somewhat like bunches of grapes, connected, as were the earliest knobs, with the same intersegmental veins. These clusters are still separate from one another.

There is a rapid extension of these blood-filled structures, and soon, in about an hour-and-a-half after their first appear-

[1] The injection tests were made with extremely fine capillary glass canulae (12 to 20 micra in diameter at the tip), which were inserted directly into the blood-filled sprout. With care the blood capillaries in their vicinity may be avoided. By using a very slight pressure extremely delicate structures may be filled without extravasation. The injections were made under the high power binocular microscope, so that the progress of the injection might be watched and controlled. This refinement in the injection technic makes possible the direct, clean-cut injection of a selected capillary, without extravasation. Our method of injection differs only in minor details from that described by B. Mozejko, "Ueber mikroskopische Injektionen nach der Methode des Prof. Heinrich Hoyer in Krakau," Zeitschrift für wissenschaftliche Mikroskopie. Bd. xxviii, H. 4, 30 März, 1912.

ance, connections between neighboring clusters may be seen. Injection at this stage with India ink shows an anastomozing plexus, connected, as before, with the intersegmental veins. Injection with silver nitrate shows distinct endothelial markings in the walls of the plexus.

During this plexus formation there is a steady extension toward the surface, and by the time anastomoses have formed between neighboring clusters, sprouts have grown to the surface and started to extend in the region superficial to the plexus and also ventralwards. It now becomes possible to study with more minuteness the changes which are going on, since these sprouts are quite superficial and are developing in a plane parallel with the surface. Observation and successive records of these sprouts in the living chick reveal a rapid extension ventrally and also anteriorly accompanied by a plexus formation. Two or three sprouts are seen to lead and soon numerous connections develop between them. Various portions of the irregular plexus thus formed enlarge and become more densely packed with blood which continues to back up from the vein. Then new sprouts grow out in advance and the same process of extension accompanied by plexus formation is repeated. If a single sprout is selected and frequent careful drawings are made, the changes are seen to be rapid and striking. The sprout becomes wider and longer. Branches appear, and they in turn increase in width and length. From a branch a connection forms with the original sprout, thus forming a loop. New branches and connections are formed making a plexus. Branches from neighboring loops or plexuses meet one another and anastomose. The several parts of the plexus are quite irregular in size. Most of the lymphatic vessels are several times as wide as a blood capillary, while some of the connections are as small as or even smaller than a blood capillary. Throughout, the blood in these new forming lymphatics is markedly darker in color than the circulating blood.

During their development the lymphatics show a tendency to avoid the blood vessels, for they spread out in the meshes of the blood vessels and at a slightly deeper level. This tendency

is shown most markedly in a space over the postero-dorsal angle of the pelvic region. Here there is a small area which is practically without a blood capillary. Into this area one of the earliest of the superficial lymphatic sprouts grows and spreads. This area has been carefully watched for several hours preceding its invasion by lymphatics. Complete blood-vascular injections have been made at this as well as at earlier stages. These studies show that, except for an occasional very fine blood capillary, this is a pure non-vascular area. Moreover, an occasional fine capillary, when present, remains during and after the invasion by the lymphatic plexus.

The injection tests at the various stages have for the sake of clearness been inserted along with the description of the appearance in the living. It should be said that the entire process, starting about an hour before the appearance of the first knobs, and including the formation of these knobs, the clusters, the plexus, and a considerable development of the superficial extensions from this plexus, may be, and several times has been watched in the same chick. Many tests have been made on other chicks which were watched until the desired stage was reached. The time which elapses between the development of the first knobs and the formation of a considerable superficial plexus is somewhat variable, but averages about three hours.

Since the stagnant blood in the interior of the lymphatics is the index on which these studies are based, it was important to determine whether the blood always fills the lymphatics to their tips. This was tested in two ways, by pressure over the part filled with blood, to see whether the blood could be forced further; and by injection. As a result of numerous tests by both of these methods it was found that, in these early stages, practically all the lymphatics, save very fine connections, are usually filled with blood. The injections failed to reveal lymphatics beyond the blood-filled structures previously seen. Hence, since the blood fills the successive extensions of the lymphatic as soon as formed, the use of the stagnant blood as an index for the study of the development of the early lymphatics is justifiable.

The development of the posterior lymph heart from the deep plexus has been traced in later stages. The plexus becomes more extensive and more dense and the vessels wider. In chicks between seven and eight days old the contractions of the heart commence while it is still in the form of a plexus. A fuller description of this later development, as well as a description of the further extension of the superficial lymphatics will be given in a later publication.

That the earliest lymphatics, those which form the primary lymph sacs, arise by the transformation of plexuses of blood capillaries, has been maintained by F. T. Lewis, Miss Sabin and by Huntington and McClure. That the extensions of these primary lymphatics into the various parts of the body may occur by the successive addition of parts of the blood capillary system which become cut off and are subsequently added to the lymphatic system, has been suggested by F. T. Lewis and maintained at one time by McClure. Our studies show that both these views are certainly incorrect. Careful observation of the blood vessels at the time just preceding and during the formation of the very first lymphatics shows that the blood vessels through which blood has circulated before the lymphatics appear are still present after the lymphatics have developed. Instead of such a transformation we have seen new blood capillaries developing almost side by side with the developing lymphatics. The same may be said of the lymphatics which extend superficially. Blood capillaries, which have been seen and recorded before the lymphatics have extended into their region, are still present after the lymphatics have reached there. This independence of the two systems is shown most strikingly in the case of the non-blood vascular area already described.[2]

If the earliest lymphatics were formed by the transformation of blood vessels, one would expect that, for a time during this

[2] It is interesting to note that the origin of the lymphatics directly from the veins, as deduced by Miss Sabin in her first paper (Amer. Jour. Anat., vol. 1), proves to be correct. The light thrown upon this subject by means of reconstruction, while of value regarding the form and position of developing lymph channels, has left us in darkness regarding their mode of growth.

transformation, complete injections of the blood vascular system would show connections between the blood capillaries and the lymphatics. We have made many such injections at each of the early stages of developing lymphatics described. The results have been invariable. The lymphatics, even from the time of their very first appearance, show no connections whatever with the surrounding blood capillary plexus. Their sole connections with the blood vascular system are the ones with the deep intersegmental coccygeal veins, and it is only through these that an injection can be made to pass from the blood vascular system over into these lymphatics. That they are not transformed blood capillaries is also shown by their irregular character, which is totally different from the pattern of the blood capillary plexus.

Another point which comes out most strikingly in these studies on living embryos is that there is no separation whatever, either in time or manner of growth, between the development of the plexus which is to form the lymph heart and that of the peripheral lymphatics. Simultaneously with the formation of the deep plexus which is to form the lymph heart, sprouts from it may be seen extending superficially and spreading out to form the peripheral lymphatics. This spreading occurs by minute stages, in which there is to be seen the gradual centrifugal extension of the lymphatics already formed. The view held by Huntington and his pupils, and by McClure, that peripheral lymphatics are formed independently of the primary lymph sacs or lymph hearts and that they have no connections with the latter until they have formed an extensive plexus, is shown by these observations to be entirely inadmissable.

The conclusions to which these observations lead us are as follows: The first lymphatics in the tail region of the chick arise as direct lateral buds from several of the main dorsal intersegmental coccygeal veins, and not by the transformation of a previously functioning blood vessel plexus. From now on the lymphatic endothelium is specific, and spreads by a steady centrifugal extension, just as has been observed by one of the authors for the lymphatics in the tad-pole's tail at a slightly

later stage in development. The buds send out processes forming clusters. From the clusters, in turn, processes are sent out which anastomose with one another, forming a plexus. Simultaneously processes grow toward the surface from the clusters, and give rise to the superficial plexus of peripheral lymphatics of the posterior part of the body. There is no essential difference between the manner of growth of the peripheral lymphatics and that of the plexus which is to form the lymph heart. In fact, the two may be considered as but portions of a single plexus of which the part nearer the veins, from which they have budded off, and with which they maintain connections, develops into the lymph heart.

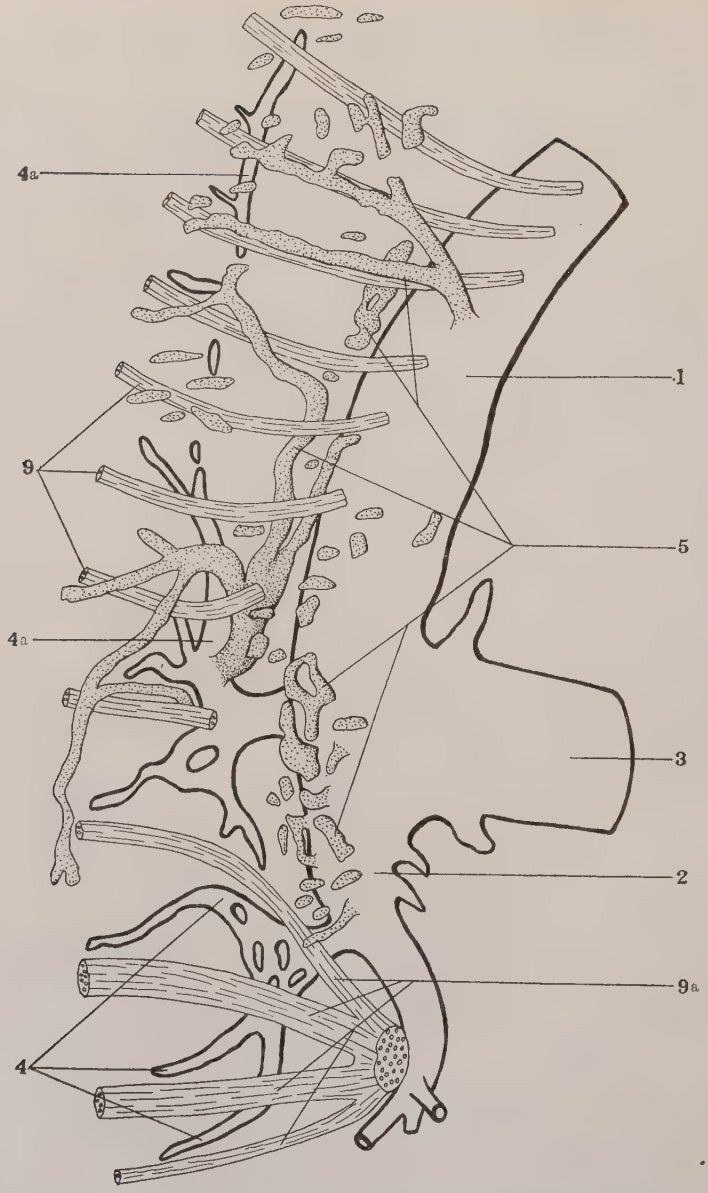

Fig. 1 Diagram drawn from a reconstruction of the veins and nerves in the cervical and upper thoracic regions of a chick embryo of five days and ten hours (13.5 mm.) Right side. *1*, Precardinal vein; *2*, postcardinal vein; *3*, duct of Cuvier; *4*, intersegmental (dorsal somatic) veins; *4a*, vertebral (dorsal somatic) vein; *5* lateral group of vascular islands and channels—veno-lymphatics; *9*, spinal (cervical) nerves; *9a*, brachial plexus.

Figure reprinted from "The American Journal of Anatomy," Volume 12, Number 4, Fig. 3, p. 479, January, 1912, A. M. Miller.

INJECTION AND RECONSTRUCTION OF THE JUGULAR LYMPH "SAC" IN THE CHICK

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TWO FIGURES

It has recently been pointed out that the present difference of opinion in regard to the mode of development of the lymphatic system is due largely to the method employed in studying the subject. Those authors who have used exclusively the method of reconstruction from cross sections, have represented the earliest lymphatics as isolated channels and spaces. From this two theories have arisen:

1. The view tentatively advanced by F. T. Lewis that the development is by the addition of portions of blood capillaries which have been cut off from the general blood vascular system; and

2. The theory championed vigorously by Huntington, McClure and others, that the extension of lymphatics is by the transformation and addition of isolated spaces in the mesenchyme.

On the other hand, the theory of centrifugal growth has been brought forward by Ranvier, Sabin, MacCallum and Hoyer, who used the method of injection as well as the study of cross sections, and by Clark who based his work on observations of living lymphatics. These authors picture the early lymphatics as continuous and state that their extension is accomplished by sprouting of the endothelium.

The limitation of the reconstruction method has been amply tested by Clark who made careful drawings of living lymphatics in the tadpole's tail and subsequently attempted to reconstruct these same vessels. Similar tests were made by Sabin on the embryo pig, to compare the method of reconstruction with that

of injection. These studies made it clear that the lymphatics cannot be completely reconstructed, for vessels which in the living embryos and in injected specimens are seen to be continuous, appear in cross sections as isolated spaces. This accounts for the discrepancies in the chief views concerning the development of lymphatics.

The recent publication by A. M. Miller[1] in The American Journal of Anatomy has given me the opportunity to compare the value of the two methods in studying the development of the jugular lymph sac in the chick, since I have succeeded in making numerous injections of the same region and stages which Miller has reconstructed. These injections show that in chicks between five and one-half and seven days the developing lymph 'sac' which appears in Miller's reconstructions as a scattered group of isolated 'islands and channels,' is in reality a dense and continuous lymphatic plexus.

The accompanying drawing, fig. 2, was made from an injected specimen of the jugular lymphatic plexus in a chick of the same size and stage as fig. 1 (reprinted from fig. 3 in Miller's paper), judging by the measurement and by the appearance of the neighboring veins. A comparison of the two demonstrates again that much of the early lymphatic system cannot be seen in cross section and therefore cannot be reconstructed.

Students of chick embryology have always encountered difficulties in determining with accuracy the various stages, on account of the evident variations in the degree of development of two embryos of the same age or measurement. From Miller's descriptions it is not possible to determine the exact stages presented in his figures. Therefore in case my figure may not represent exactly the same stage as Miller's fig. 3, it may also be compared with his fig. 1, which shows a chick of the same age as my specimen, or with his fig. 4 which shows a reconstruction of an older embryo. In any case the contrast between the continuous plexus disclosed by injection and the isolated islands shown by reconstruction is most striking.

[1] A. M. Miller, "The Development of the Jugular Lymph Sac in Birds," American Journal of Anatomy, Vol. 12, No. 4, p. 473, Jan. 1912.

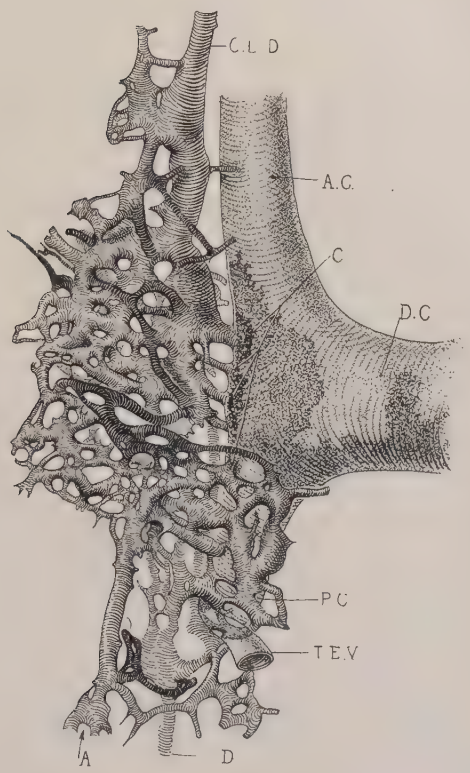

Fig. 2. Lateral view of jugular lymphatic plexus of the right side in a chick embryo incubated five days and twenty hours, measuring 14 mm., greatest length, after fixation. By means of a very fine glass canula (about 15 micra in diameter at the tip) dilute India ink was injected, under the binocular mircoscope, directly into one of the superficial lymphatics of the body wall, between the arm and leg. From here the ink filled the deep jugular lymph plexus and from it a few granules entered the vein through five connections (*C*). The drawing was made with the assistance of a camera lucida with a Zeiss binocular, oculars no. 4, objectives A_3. Enlarged 50 diameters. *A*, vessel connecting the superficial lymphatics, into which the injections were made, with the jugular lymphatic plexus; *A.C.*, anterior cardinal vein; *P. C.*, posterior cardinal vein; *D. C.*, duct of Cuvier; *C.*, communications between jugular lymphatic plexus and vein; *D.*, deep lymphatic, *T. E. V.*, thoraco epigastric vein; *C. L. D.*, cervical lymphatic duct.

Compare with Fig 1, which is a reprint of Miller's diagram drawn from a reconstruction of this region in a chick of approximately the same stage.

I have also made injections in chicks of seven days,—the stage shown in fig. 6 of Miller's paper. Here instead of the 'sac' described by Miller, the injected specimens show that an extensive plexus is still present. The vessels composing it are larger than in the younger chicks, and Miller has probably figured the largest of these, which in my injections is seen to be continuous with a large lymphatic duct lying next the jugular vein and extending the whole length of the neck. No doubt this is Miller's lymph 'sac' of the seven-day chick.

I have not yet studied this region in chicks older than eight days, but the injections of chicks between seven and eight days confirm Mierzejewski in his descriptions of a 'lymphatic plexus' in contrast to the 'sac' figured by Miller.

I may add that in all the chicks injected between five and one-half and eight days I was able to demonstrate a connection with the venous system. In the younger chicks (see figure) the connections are more numerous, but subsequently the number is reduced to one or two. I should therefore seriously question Miller's statements in regard to the separation of the sac from the vein and its subsequent reunion with it.

I do not claim that any of my injections, including that shown in the figure, are entirely complete but, as far as they go, they are positive. They reveal the presence of a definite continuous lymphatic plexus in a region where only isolated spaces can be demonstrated by the reconstruction method.

ON A DOUBLE FENESTRAL STRUCTURE IN EMYS

B. W. KUNKEL

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TWENTY-FOUR FIGURES

Although the comparative anatomy and development of the sound-transmitting apparatus of reptiles has been extensively studied, the chelonians have received attention in this connection only from Fuchs ('07), Noack ('07), Bender ('11), and very briefly from the present writer ('11). Rathke as early as 1839 observed the continuity of the columella and hyoid arch in snakes. The comparative anatomy of the lacertilian columella auris has been exhaustively studied by Versluys ('03) and was shown by him to be composed of two morphologically distinct parts, a median segment, the stapes, closing the fenestra vestibuli, and a lateral segment, the extracolumella, which remains cartilaginous through life and whose outer end is attached to the tympanic membrane. The independence in origin of columella and otic capsule has not been recognized, however, by all investigators although at the present time the evidence is quite convincing that the columella auris is entirely extra-capsular in origin. In Lacertilia, according to Versluys, the extracolumella apparently takes part in the completion of the hyoid arch and in fact it has been termed the hyostapes by Hoffman ('89) in contradistinction to the otostapes, which that investigator regarded as of labyrinthine origin.

In Testudo, according to Bender ('11), the columella is united with the hyale by means of the interhyale and the extracolumella lies wholly external to the arch which is made up of the cornu hyale, interhyale, and columella. Thus the sound-transmitting apparatus of the chelonians differs from that of the lacertilians,

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principally in the relation of the extracolumella to the hyoid arch. That the extracolumella is not a portion of the original arch in the chelonians is evident from the fact that it is, especially in the early stages, considerably behind the other parts of the arch in its degree of chondrification. Later in development, as I have found in Emys lutaria, a processus inter-hyalis, which is as highly chondrified as the extracolumella itself, a d which represents the separate interhyale fused to the extracolumella, may be recognized in certain stages on the medio ventral angle of the extracolumella. Regarding the homologies of the columella of the Chelonia, there has been some doubt, as the connection of the same with the hyoid was not established before Bender's recent paper. My own work on Emys which has been extended since my first paper appeared confirms Bender's results on Testudo.

In the present paper I wish to call attention especially to a condition met within several embryonic stages of Emys lutaria, which has not been noted hitherto in the Reptilia, and in which respect Emys resembles the Amphibia. This is the indication of a separate chondrification in the ventral wall of the otic capsule posterior and median to the fenestra vestibuli which is possibly homologous with the operculum of the Urodela. At present it is impossible to confirm the homology absolutely and accordingly I denote it throughout the present paper as stapes inferior.

Kingsbury and Reed ('09) in their extensive work on the columella auris in Amphibia have shown that in the development of the Urodela there are recognized two more or less distinct cartilaginous structures which occupy the fenestra vestibuli, the columella, which possesses a suspensorial connection, and the operculum which lacks the same. The latter segments off from the wall of the capsule itself while the former lies dorsal, or external, and anterior to the latter and develops independently from a center external to the capsule, as Killian ('90) first described in Amblystoma and as was confirmed by Miss Platt ('97) in Necturus, and by Kingsbury and Reed ('09) in Cryptobranchus, Spelerpes, and Plethodon. The columella typically possesses a stilus which extends laterally from the fenestra vesti-

buli between the vena capitis lateralis above and the arteria carotis interna below. The relation of the stilus to the nervus facialis is somewhat variable among the Amphibia although Kingsbury ('03) has shown that this relationship of nerve and columella is not of fundamental importance in establishing the homology. In Necturus, Proteus, and Typhlomolge, the ramus jugularis nervi facialis passes above the stilus while in all the other forms in which a stilus is present, the nerve lies ventral to it. In Emys the columella passes ventral to the entire hyomandibular trunk and not to only a part of it as is the case in the Urodela. This, however, does not seem to be an important difference between the columellae of the two groups. Besides this, its origin external to the otic capsule, its position ventral to the vena capitis lateralis and dorsal to the arteria carotis interna, point toward the morphological similarity of the two. The relations of the external end of the columella, however, offer some difficulties. In Emys the columella is connected with an extracolumella and an interhyale, the latter of which Bender has shown connects it with the hyoid arch. In addition to this the extracolumella is continuous anteriorly and dorsally with the quadratum in early stages. In the Urodela, on the other hand, according to Kingsbury and Reed, the primary relationship of the lateral end of the columella is with the squamosum, an extracolumella not being present.

The structure which I designate as stapes inferior in Emys agrees with the operculum of the Urodela in: (a) its relation to the fenestral plate of the columella, (b) its origin from the otic capsule, (c) its tendency to be confluent with the otic capsule caudally, and (d) its relation at an early stage to the ductus perilymphaticus. Kingsbury and Reed on page 605 give as the criteria of the operculum in Urodela its "lack of connection with the skeleton outside of the ear capsule and the attachment of M. opercularis." The absence of this muscle in the Chelonia prevents the application of this second criterion.

At the earliest stage which I have studied, in which the imperfectly differentiated carapace measured about 4.7 mm. in length, the cartilages of the skull are laid down in the most advanced

portions simply as early prechondrium. At this stage the otic capsule is represented by a mass of mesenchyme in which the matrix characteristic of prechondrium is not yet present, although the nuclei are somewhat crowded together, as occurs in undifferentiated mesenchyme before chondrification takes place. The process of chondrification apparently begins at a point toward the posterior end of the capsule in the region immediately dorsal and posterior to the future fenestra vestibuli, surrounding the external opening of the canalis glossopharyngeus in which the nervus glossopharyngeus lies in its passage through the otic capsule. In its dorsal and median portions, the capsule is imperfectly marked off from the surrounding mesenchyme. The connection of the capsule with the planum basale does not extend in front of the nervus facialis. An earlier stage in which the separation between these two parts is complete was not available in the series studied although there are indications of this in my youngest specimen (fig. 1). The fusion apparently begins somewhat posterior to the middle of the capsule and extends more rapidly anteriorly than posteriorly.

In my youngest embryo the columella exhibits a much more advanced stage of chondrification, especially at its lateral end, than the otic capsule. Its median end presses slightly against the lateral wall of the cochlear portion of the capsule at a point corresponding to the future fenestra vestibuli which has not yet formed (fig. 2). In this and the succeeding stage the more advanced chondrification of the columella is quite distinct from that of the capsule. The extracolumella is composed of a mass of less highly differentiated mesenchyme which is situated at the lateral end of the columella and is continuous anteriorly and dorsally with the blastem of the palatoquadratum. On the outside of the body it is evident as a strong convexity immediately ventral to the external opening of the hyomandibular cleft (fig. 2). Midway between the distal end of the columella above and that of the hyoid arch below, and so completing the curve between these two parts, is the interhyale. This has the form of an ovoid mass of prechondrium (fig. 3), as Bender has already shown. The columella auris and interhyale are to be

ABBREVIATIONS

a.c.c., arteria carotis communis
a.c.i., arteria carotis interna
c.b., crista basipterygoidea
c.c., cavum cochleae
ch.d., chorda dorsalis
col., columella auris
c.p., crista parotica
c.s.l., canalis semicircularis lateralis
c.t., chorda tympani
d.p., ductus perilymphaticus
ec., extracolumella
f.h., foramen anterius spino-occipitale
f.m., fissura metotica
g.a., ganglion acusticum
g.g., ganglion glossopharyngeum
g.gen., ganglion geniculi
h., hyale
ih., interhyale
lab., membranous labyrinth
M.c., Meckel's cartilage
o., stapes inferior
ot.cap., otic capsule
p.b., planum basale
q., quadratum
r.s.t., recessus scalae tympani
s., squamosum
v.c.l., vena capitis lateralis
1., first branchial cleft
VII, ramus hyomandibularis nervi facialis
VII–IX, ramus communicans nervi facialis ad nervum glossopharyngeum
VIII, nervus acusticus
IX, nervus glossopharyngeus
X , nervus vagus

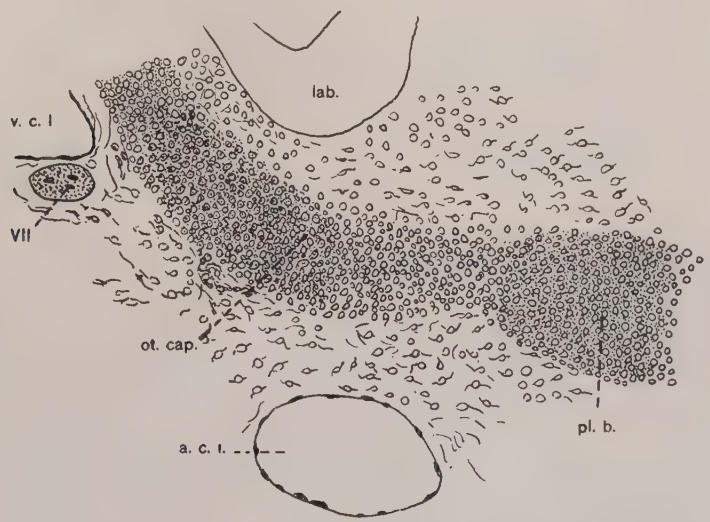
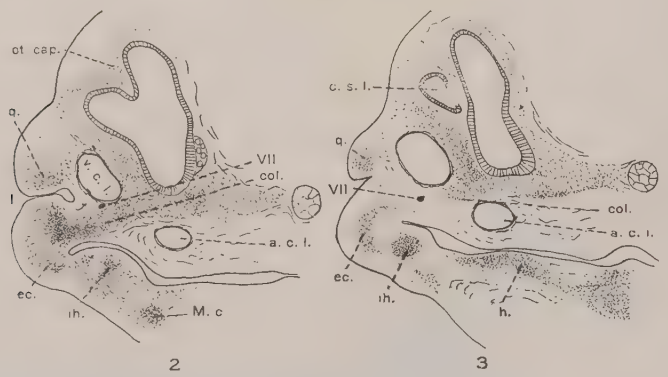


Fig. 1 Transverse section of an embryo having a carapace-length of 4.7 mm., through the ventral portion of the otic capsule in front of columella auris, showing the line of union between planum basale and otic capsule. × 200.

looked upon as derivatives of a previously continuous hyoid arch lying between the first and second visceral clefts. The extracolumella, according to Bender, is a secondary addition to the most lateral portion of the hyoid arch. In respect, then, to the relation of columella, extracolumella, and hyoid, there is essential agreement between the Chelonia and Lacertilia.

The relation of the nerves and blood vessels to the structures in question is the same as was described by Noack ('07). The vena capitis lateralis is a large vessel which extends lateral to the otic capsule immediately dorsal to the ganglion vagi posteriorly and lodged in the shallow groove which marks the lateral



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Figs. 2 and 3 Transverse sections through the otic capsule of an embryo having a carapace-length of 4.7 mm. The section in fig. 3 is 105μ posterior to that of fig. 2. $\times$ 40.

surface of the capsule ventral to the prominentia canalis semicircularis lateralis. The arteria carotis interna lies ventral and lateral to the otic capsule and is separated from the aforementioned vein by the columella auris. The ramus hyomandibularis n. facialis extends in a posterior direction from the ganglion geniculi alongside of the otic capsule and dorsal to the columella. At the level of the columella, or immediately behind it, the chorda tympani is given off in a horizontal direction laterally. This branch then bends forward and ventrally to the mandible. After giving off the chorda tympani, the ramus hyomandibularis continues posteriorly and gives off the ramus communicans n.

facialis ad glossopharyngeum and then bends ventrally to the muscles which it supplies.

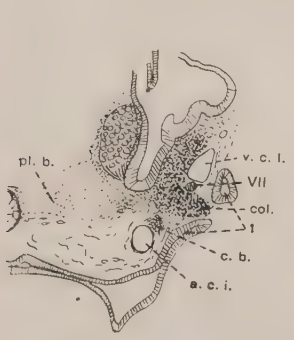
At a later stage (an embryo with a carapace-length of 5.2 mm.) when chondrification has progressed further, the fenestral plate of the columella comes to rest against the lagena so that the fenestra vestibuli is already formed although the fenestral plate fills it completely. The distinction between the fenestral plate and the capsular wall becomes more pronounced with age on account of the relatively greater rapidity of chondrification of the columella. Anteriorly and ventrally, as well as antero-dorsally, there is a distinct line of demarcation between the capsular wall and the fenestral plate although postero-dorsally there is a more gradual transition between the two structures. Immediately medial to the ventral margin of the fenestral plate and forming the floor of the lagenar portion of the capsule is a space which is quite distinctly marked off from the lateral margin of the planum basale on the one hand and the columella on the other. This area is characterized by its cells with smaller nuclei crowded together in contrast on the one hand to the larger and more widely separated ones of the planum basale which also exhibit a concentric arrangement, and on the other to the more advanced chondrification of the fenestral plate of the columella.

The condensed mesenchyme filling this space between the fenestral plate and the planum basale is somewhat anterior to the future stapes inferior which has not yet become differentiated. From the lateral margin of this space and extending nearly as far posteriorly as the fenestral plate of the columella is a distinct ridge which represents the crista basipterygoidea projecting ventro-laterally above the arteria carotis interna.

At the next stage (an embryo having a carapace-length of 7 mm.) the relation of these parts is essentially the same as has been described, but because of the more advanced stage of chondrification the boundaries of the various cartilages are more distinct. There are, however, several changes worthy of note.

The otic capsule becomes continuous with the planum basale by both a pre- and postfacial commissure. The lateral margin

of the planum basale extends freely as a ventro-laterally projecting ridge of less highly differentiated prechondrium, the crista basipterygoidea, which in passing posteriorly toward the region of the foramen faciale becomes separated from the planum



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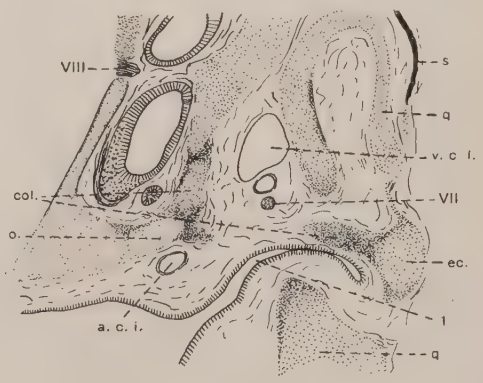

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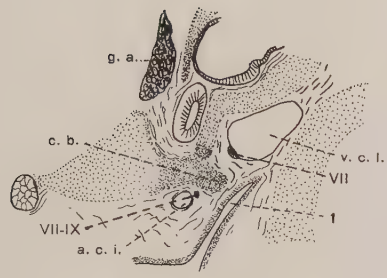
Fig. 4 Transverse section through the otic capsule of an embryo having a carapace-length of 5.2 mm., showing the extension of the fenestral plate of columella dorsally and the crista basipterygoidea. × 40.

Fig. 5 Transverse section through the otic capsule of an embryo having a carapace-length of 8 mm., showing the relations of columella, stapes inferior, first branchial cleft, and extracolumella. × 40.

basale and continues as a longitudinal rod parallel to the lateral margin of the planum basale and connected with it by a mass of dense mesenchyme (fig. 22). Posteriorly this ridge comes to lie more nearly horizontally and in the region immediately in front of the columella it fuses with the floor of the otic capsule which is here quite distinctly separated from the planum basale (fig. 6).

Figs. 6 to 13 A series of transverse sections through the posterior portion of the otic capsule of an embryo having a carapace-length of 7 mm. The crista basipterygoidea terminates in front of the stapes inferior. The columella extends medially against the membranous labyrinth and lies ventral to the vena capitis lateralis and ramus hyomandibularis nervi facialis and dorsal to the arteria carotis interna and ramus communicans nervi facialis ad nervum glossopharyngeum. In fig. 10 the columella and stapes inferior are seen alongside of each other, while caudal to this section (fig. 11, et seq.) only the latter structure is visible. × 40.



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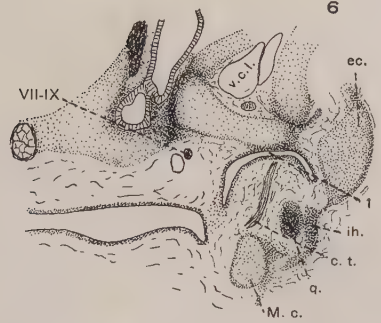

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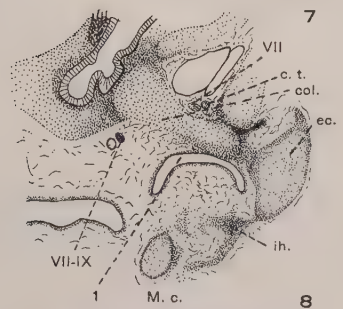

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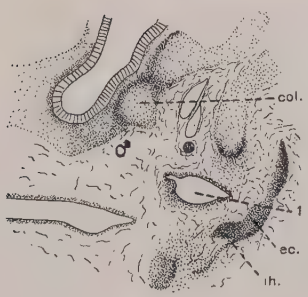

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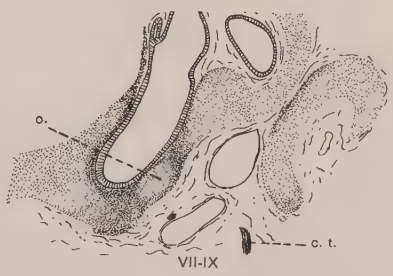

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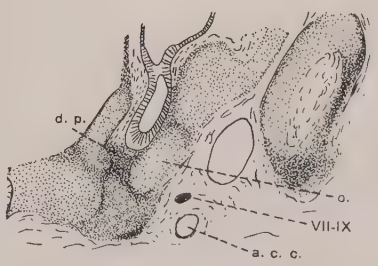

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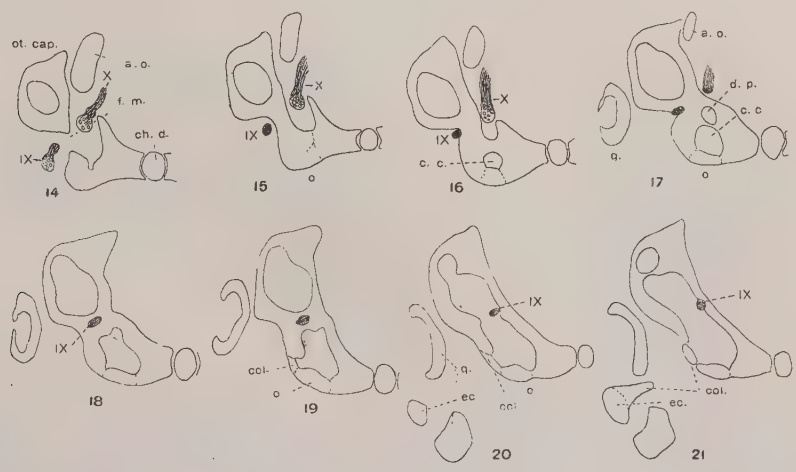
The floor of the capsule, beginning a short distance in front of the columella is demarcated from the planum basale by being less highly chondrified than the latter, as in the previous stage, and by being constricted off from it by a shallow groove on its ventral side. Further caudally the constriction disappears and the chondrification exhibits further development so that the planum basale apparently extends as far as the ventral margin of the fenestral plate of the columella. From the outer margin of this plate of younger prechondrium the posterior end of the crista basipterygoidea extends in a horizontal direction immediately beneath the columella where it disappears (fig. 6). A few sections behind the end of the crista basipterygoidea, the capsular wall immediately ventral to the fenestral plate of the columella exhibits a retarded chondrification and further caudally becomes segmented from the planum basale (fig. 10) resembling closely the operculum of the urodelan otic capsule. The stapes inferior at this stage is somewhat lens-shaped with the ventral edge in front thicker than the dorsal margin. Further caudally it becomes symmetrical. It lies with its median face close against the lagena immediately ventral and posterior to the columella. Accordingly, it has a position slightly inclined to the vertical. It rests with its ventral edge upon the floor of the capsule which is joined medially to the lateral margin of the planum basale and differentiated from it by its younger condition. Posteriorly and dorsally it becomes confluent with the wall of the capsule, while ventrally it remains quite distinct from the condensed mesenchyme lying between it and the planum basale.

Posterior to the stapes inferior the ventro-lateral capsular wall rolls over medially at its ventral edge to form a kind of trough in which the most posterior portion of the sacculus rests (fig. 13). Its free median edge is separated by a narrow space from the median wall of the capsule in which space the first trace of the ductus perilymphaticus is seen (figs. 12 and 13).

The ventro-lateral wall of the capsule, which is continuous with the stapes inferior, therefore comes to lie interposed between the lagena dorsally and the mass of condensed mesenchyme

ventrally which further forward forms the floor of the capsule. A few sections further back (fig. 24) the portion of the capsular wall which is continuous anteriorly with the stapes inferior is seen ventral to the nervus glossopharyngeus and dorso-lateral to the recessus scalae tympani within which a portion of the ductus perilymphaticus lies.

The mass of tissue lateral to the planum basale and ventral to the stapes inferior is apparently formed from the mesenchyme of the surrounding regions by actual condensation. It extends



Figs. 14 to 21 Transverse sections through the posterior portion of an embryo having a carapace-length of 9 mm. showing the relation of nervi glossopharyngeus and vagus, ductus perilymphaticus, columella, and stapes inferior. The sections are in order from posterior to anterior. × 20.

beyond the external surface of the neighboring cartilages as if overflowing and spreading around their margins (fig. 12); still further posteriorly at the extreme anterior end of the fissura metotica, this mass of condensed mesenchyme forms a kind of knob projecting from the lateral margin of the planum basale in a dorso-lateral direction and apparently partially occluding the fissure from its anterior end and enclosing below and on the side the recessus scalae tympani. The musculus collocapitis brevis is evident at this time simply as a mass of much

concentrated tissue occupying the ventro-lateral angle of the planum basale and stretching caudally.

In an embryo having a carapace-length of 8 mm. the stapes inferior is still separate from both the planum basale and the otic capsule. The fenestral plate of the columella is well separated from the margins of the fenestra especially along its ventral margin, but even along the upper margin where the continuity between the fenestral plate and the capsule is more apparent, the differentiation is appreciable (figs. 14–21). On the ventral

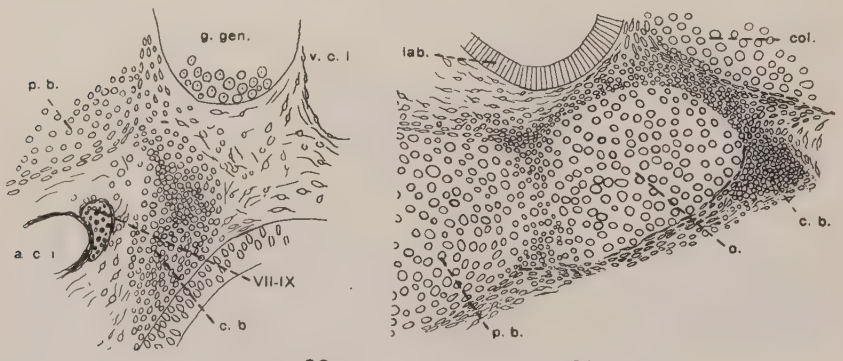


Fig. 22 Transverse section of an embryo having a carapace-length of 7 mm. showing the separation of the planum basale and crista basipterygoidea. × 200.

Fig. 23 Transverse section through the floor of the otic capsule of an embryo having a carapace-length of 8 mm. showing the anterior end of stapes inferior, crista basipterygoidea, and columella. × 200.

side of the fenestral plate there is a wide gap between it and the stapes inferior (fig. 5).

The crista basipterygoidea at this stage has grown further caudally beyond the front margin of the columella as a cylindrical rod, and is separated slightly from the capsule, lying lateral to the dorsal margin of the stapes inferior (fig. 5), which has extended itself further anteriorly than in the previous stage.

The stapes inferior at this stage exhibits on its inner surface a decided convexity so that it is strongly lens-shaped, but because its upper margin is thinner than the fenestral plate there is left a recess between it and the fenestral plate (fig. 19).

After this stage of development is reached the stapes inferior apparently fuses indistinguishably with the otic capsule and its identity is lost.

In summing up, then, the results of this paper there is, at certain stages of development of Emys lutaria, a segmentation

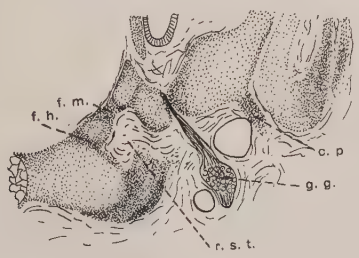


Fig. 24 Transverse section through the posterior end of the ventral portion of the otic capsule of an embryo having a carapace-length of 7 mm. This section is the most posterior of the series of sections in figs. 6 to 13. The section shows the relation of nervus glossopharyngeus, otic capsule, recessus scalae tympani and fissura metotica. × 40.

of the wall of the otic capsule in the region immediately ventral, or medial, to the fenestral plate of the columella which forms a plate that is continuous caudally with the capsule and at an early stage lies lateral to the ductus perilymphaticus, as has been described for the operculum in Urodela.

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OBSERVATIONS CONCERNING THE COMPARATIVE ANATOMY OF THE DIENCEPHALON

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FOUR FIGURES

The purpose of this article is to describe briefly in the diencephalon of the lemur and the cat certain cell groups already described by me in the human. It will be necessary to give a short statement of the different methods of dividing the diencephalon into nuclei, as based upon histological evidence.

A division of the diencephalon based upon histological evidence will vary according to the method of interpretation. Two methods of division may be recognized:

1. The *topographical,* by which the diencephalon is divided into many fields termed (in many cases erroneously) nuclei. These fields are for the most part determined by the splitting up of the gray matter by fiber masses. Such a division according to the gross, mechanical grouping of gray matter may be obtained from Nissl preparations as readily as those of Weigert-Pal, if the cell preparations are studied merely as the positive of the Weigert-Pal negative; unless the cell character is considered, the correspondence of the Nissl picture to that of the fiber preparation is no indication of the correctness of the resulting division. When so interpreted the two pictures must necessarily correspond.

2. The second method of division is that employed by me in my monograph "Über die Kerne des menschlichen Diencephalon," and divides the diencephalon into *primary nuclei.* A primary nucleus I have defined as a more or less circumscribed group of cells having an identical (histological) character. With our present knowledge we are justified in setting aside a group

of cells as a primary nucleus, only when the histological character of these cells differs markedly from that of the surrounding cells. And if we take into consideration the fact that certain purely mechanical influences (such as compression by dense fiber masses) may change the histological picture of a cell, we are justified in assuming: that *a fairly well circumscribed group of cells having an identical histological character* (primary nucleus) *has a definite primary function*, and further, that *two primary nuclei whose cells show a marked difference in histological character possess a different primary function.* To sum up: The division into primary nuclei depends upon (a) an entire indifference to the splitting up of cell masses by bundles of fibers, and (b) the bringing together of cells with an identical histological character to constitute a primary nucleus, even when these cells are intermingled with those of one or more other primary nuclei. The purpose of such a division is: (a) to prepare a basis for finer experimental and pathological study, and (b) by the location of certain definite cell types to enable us to state their function, even when these cells are not directly accessible to experiment.

In making a comparative study of the diencephalon of different animals, one is impressed with the great difference in the structure and grouping of the cells. This difference is greatest in the thalamus, and since its development depends upon that of the cerebral cortex, these dissimilarities are to be expected. To give an adequate description of the relations between the thalamus of the cat and of man, would require a careful study of several intermediate forms. But the hypothalamus is an older part of the diencephalon, and consequently we find here better differentiated cell groups, which show comparatively slight variations in different animals. Therefore it is possible to compare certain primary nuclei in the hypothalamus of the cat and man, with the aid of only the lemur as an intermediate form. Moreover the recent article of Friedemann on cercopithecus affords further material for such a comparison.

In addition to the five series of the human diencephalon, which have been described in a previous article, the present description is based upon three complete transverse series of

the cat, and one of the lemur. All series were stained with toluidin-blue.

The human hypothalamus I have subdivided into the following primary nuclei: (1) Corpus hypothalamicum, (2) Nucleus medialis corporis mammillaris, (3) Nucleus intercalatus corporis mammillaris, (4) Nucleus mammillo-infundibularis, (5) Nucleus paraventricularis hypothalami, (6) Substantia reticularis hypothalami, (7) Substantia grisea ventriculi tertii. All of these nuclei may be identified in the cat and lemur, although the picture is less clear than in the human.

As some of them have been described only once (in cercopithecus) since their existence was pointed out by me in my monograph on the human diencephalon, it seems advisable to mention certain observations, although a thorough description must await the preparation of further material.

The corpus mammillare in the cat and in the lemur presents the same nuclei described by me in man, and more recently by Friedemann in cercopithecus:

1. The ganglion mediale corporis mammillaris, which is composed of small cells and occupies the greater part of the mamillary body, is well known and may at once be dismissed.

2. The nucleus mammillo-infundibularis corresponds in part probably to the lateral ganglion of the authors. But in addition to forming the lateral portion of the mammillary body, its cells, which through their histological character can be distinguished from the surrounding cells, extend in a dorsal and oral direction into the region of the infundibulum. The existence of this nucleus has been confirmed by Friedemann, who adopts for it the name here given. This nucleus is less well developed in the cat and the lemur than in man, and does not stand out so distinctly.

3. The nucleus intercalatus corporis mammillaris is in the cat and lemur relatively better developed than in man. On the other hand it is not always so easily distinguished from the lateral ganglion (nucleus mammillo-infundibularis). This nucleus consists of a round or oval group of cells, which in its caudal portion lies on the latero-ventral margin of the corpus mam-

millare; it extends in a dorso-oral direction between the medial and lateral ganglion. Whether the nucleus accessorius, which Kölliker has described in man, is identical with my nucleus intercalatus, is doubtful, since according to Kölliker the cells of the nucleus accessorius are smaller than those of the other two groups of the corpus mammillare; the cells of the nucleus intercalatus are on the contrary larger than those of the medial ganglion. Since Kölliker has not given any accurate description of these cells, nor of the extent of this cell group, it seems probable that he has described one of the islands of cells often mechanically cut off by a fiber mass from the medial ganglion. Friedemann describes a nucleus intercalatus in cercopithecus, and adopts this name, after discussing the nucleus accessorius of Kölliker.

An exceedingly sharply defined group of cells has been described by me in man under the name of 'nucleus paraventricularis hypothalami'. It consists of a compact column of large deeply staining cells, and lies near the third ventricle. It is well developed in the cat and lemur. Under the same name this nucleus has been recently described in cercopithecus by Friedemann. It probably corresponds to the nucleo subventricular described by Cajal in the rabbit. Possibly the nucleus paraventricularis hypothalami corresponds to the nucleus subcommissuralis described by Ziehen in marsupials.

Under the name of substantia reticularis hypothalami may be classed certain cells of diverse histological character which are distributed more or less diffusely throughout the hypothalamus. It seems inadvisable to attempt a thorough description of these cells until more material is available. But one point must be mentioned, to which I have already called attention in my monograph on the human diencephalon. The hypothalamus of the cat and lemur, just as in man, is characterized by containing the only cells in the entire diencephalon of a motor structure. These cells are similar to the motor cells of the anterior quadrigeminal body, and form a direct continuation of these cells into the hypothalamus. Of course it is not probable that these cells are part of peripheral neurones; the modified histological

picture is against this. But it is highly probable that they are more or less directly associated with peripheral motor neurones. This view is strengthened by the fact that in the substantia reticularis of the hypothalamus numerous transition types of cells occur, from those entirely devoid of motor structure to such as can only with difficulty be distinguished from genuine peripheral motor cells. Another very significant fact is that *no trace of motor structure can be detected in any of the cells of the thalamus, epi-, or metathalamus, which are exactly those portions of the diencephalon which are known to contain sensory centers.*

CONCLUSIONS

1. Among the cell groups of the hypothalamus of the cat and the lemur the following primary nuclei may be identified with similarly named nuclei described in my monograph on the human diencephalon: (1) Ganglion mediale corporis mammillaris, (2) Nucleus mammillo-infundibularis, (3) Nucleus intercalatus corporis mammillaris, (4) Nucleus paraventricularis hypothalami, (5) Substantia reticularis hypothalami.

2. Cells of an undoubted motor structure occur in the hypothalamus of the cat, lemur and man. Such cells are entirely absent from the other divisions of the diencephalon.

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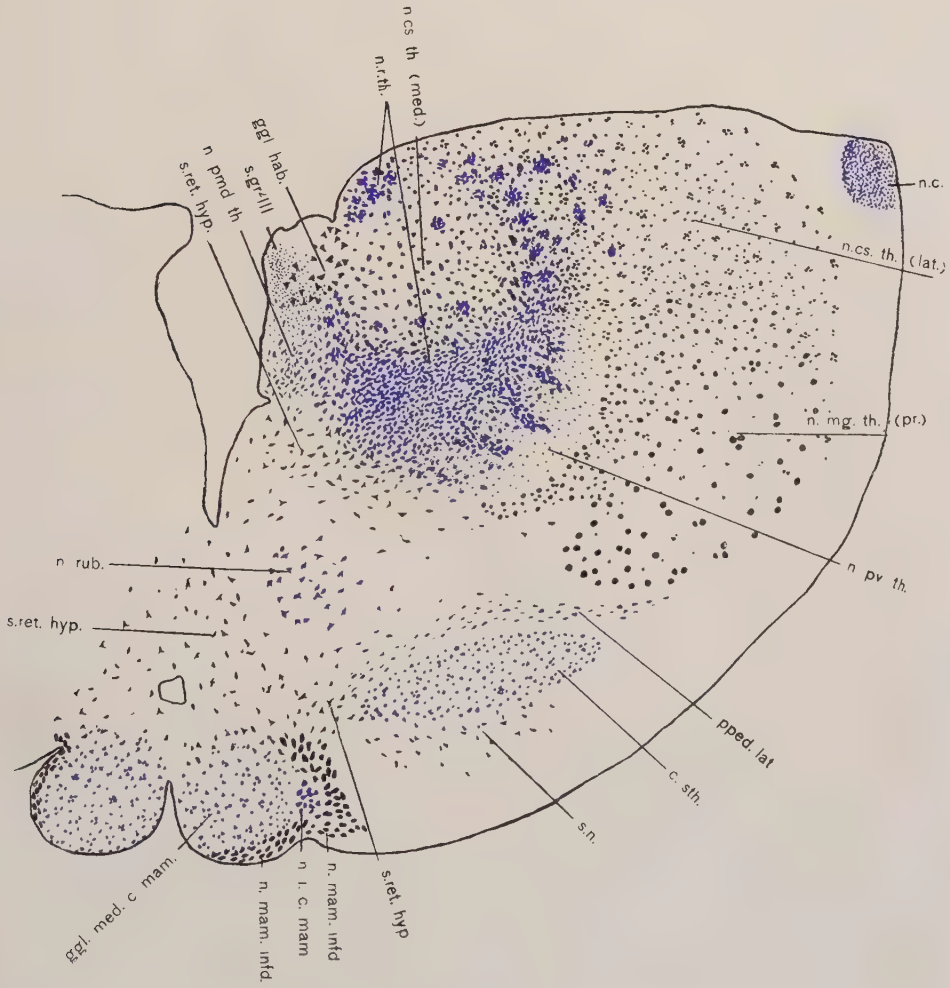
EXPLANATION OF FIGURES

The figures represent cross sections through the human diencephalon. Drawings of the cat and the lemur have not been included, since they would necessitate a more definite expression of facts than would be justified by the amount of material at present available. The outlines of the drawings were made with aid of the Edinger drawing apparatus, and the details filled in under control of the microscope.

The original magnification was 10 diameters. Fig. 1 has been reduced to 3.75, and figs. 2 to 4 to 3.3 diameters; the magnification of the cells is much greater. The drawings show merely the position of the various nuclei, and the arrangement of the cells within each nucleus.

ABBREVIATIONS

com.med., comissura media
c.sth. corpus subthalamicum
f. fornix
ggl.hab., ganglion habenulae
ggl.med.c.mam., ganglion mediale corporis mammillaris
g.o.b. ganglion opticum basale (so called)
n.c., nucleus caudatus
n.cs.th., nucleus communis thalami
n.cs.th. (*dors.*), nucleus communis thalami, pars dorsalis
n.cs.th. (*lat.*), nucleus communis thalami, pars lateralis
n.cs.th.(*med.*), nucleus communis thalami, pars medialis
n.i.c.mam., nucleus intercalatus corporis mammillaris
n.mg.th.(*arc.*), nucleus magnocellularis thalami, pars arcuata
n.mg.th.(*pr.*), nucleus magnocellularis thalami, pars principalis
n.mam.infd., nucleus mammillo-infundibularis
n.pmd.th., nucleus paramedianus thalami
pped.lat., nucleus peripeduncularis lateralis (Jacobsohn)
n.pv.hyp., nucleus paraventricularis hypothalami
n.pv.th., nucleus parvocellularis thalami
n.r.th., nucleus reuniens thalami
n.rub., nucleus ruber
s.gr^1 III, substantia grisea ventriculi tertii, pars inferior
s. gr^2 III, substantia grisea ventriculi tertii, pars superior
s.n., substantia nigra
s.ret.hyp., substantia reticularis hypothalami
t., cells of the telencephalon
tr. th. mam., tractus thalamo-mammillaris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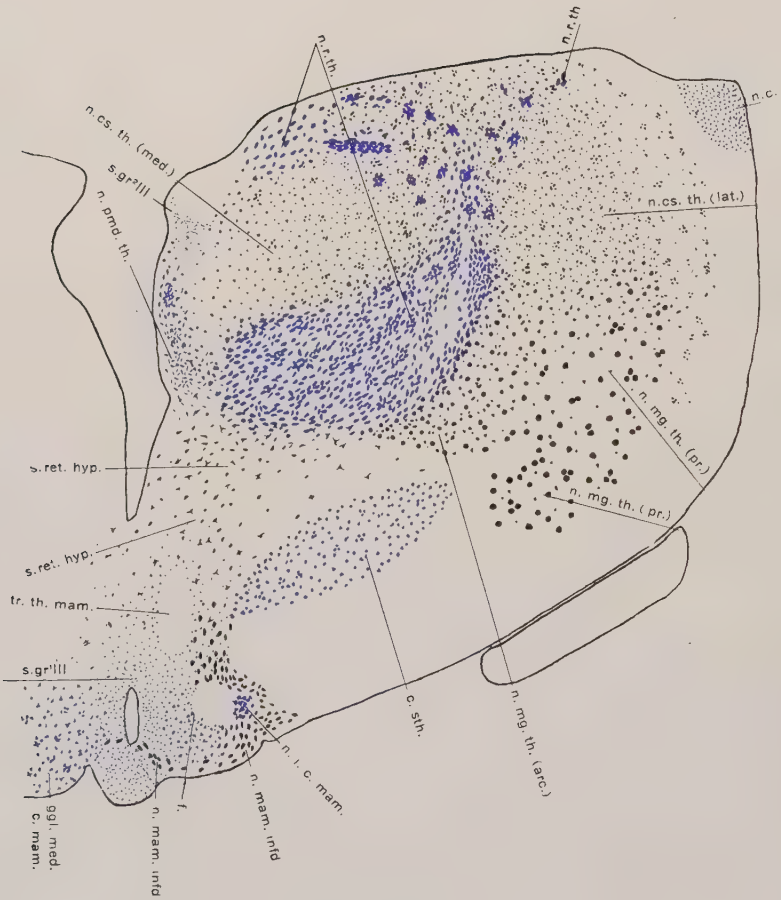
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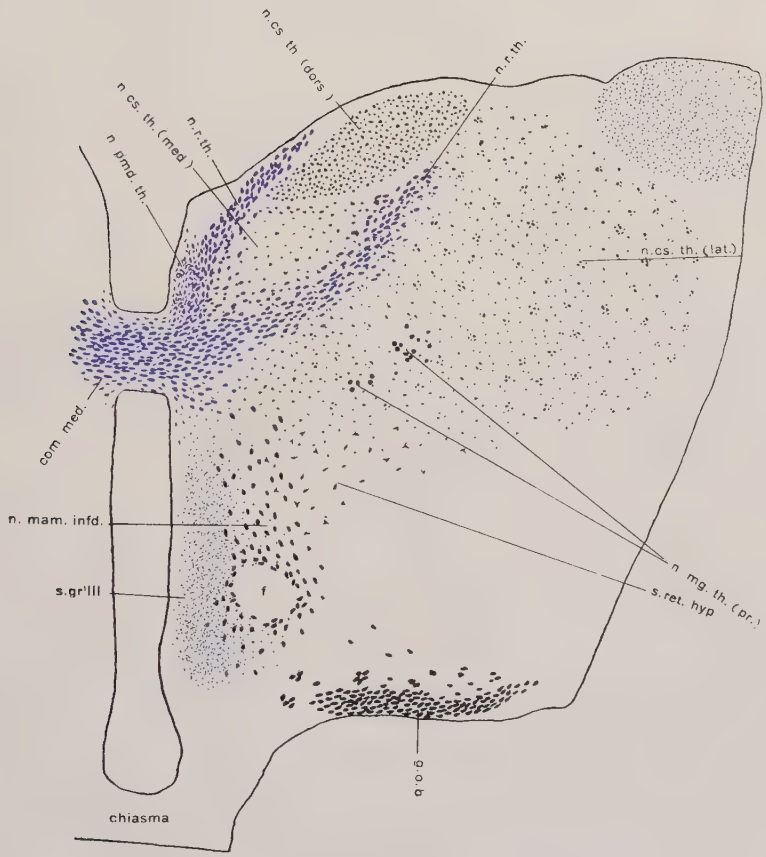
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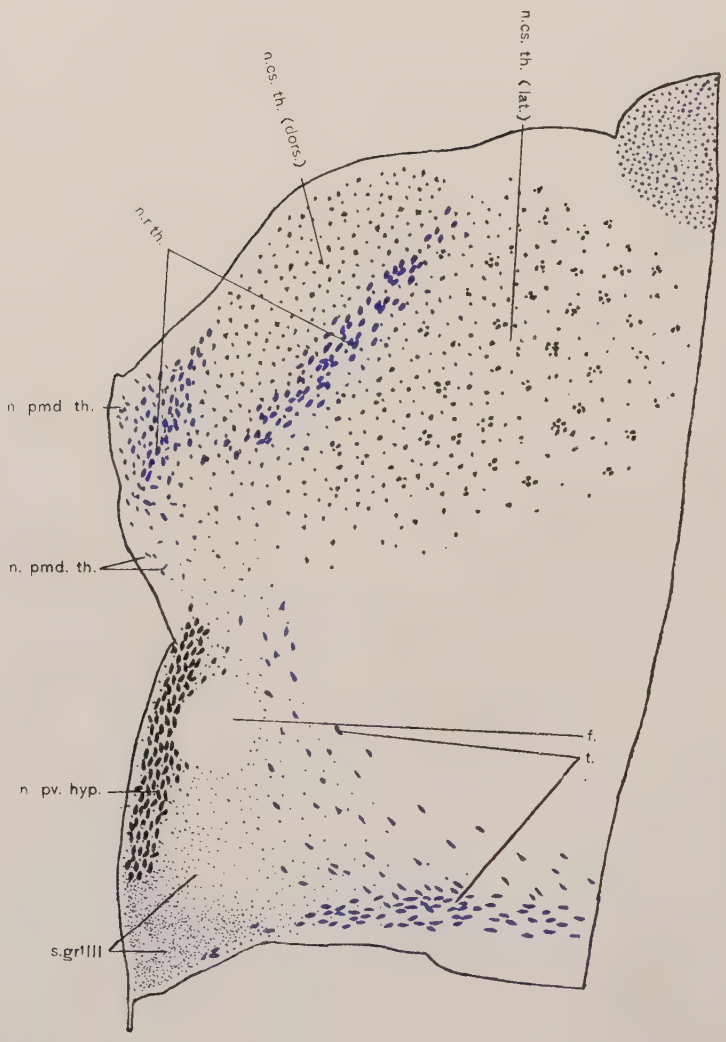

4

ANEURYSM OF THE MEMBRANOUS SEPTUM PROJECTING INTO THE RIGHT ATRIUM

FRANKLIN P. MALL

From the Anatomical Laboratory of The Johns Hopkins University

THREE FIGURES

While studying the anatomy of the heart there came into my possession quite a rare specimen in which there was a cystiform aneurysm at the junction of the aorta with the left ventricle. It involved the membranous septum, burrowed into the anterior part of the medial cusp of the tricuspid valve, and projected into the right atrium (fig. 1). The heart was greatly hypertrophied, the muscle being very well developed. The septum of the ventricle was displaced to the left, that is, it had not shifted sufficiently to the right in development, thus possibly accounting for the aneurysm.

In his great work upon the defects of the septum[1] Rokitansky considers anomalies of this kind as due to endocarditis, a view which is not entertained to so extreme a degree by v. Buhl in a subsequent study.[2] From a perusal of the literature, which is well given by these two authors, as well as from a study of the development of the membranous septum of the human heart, I am inclined to the opinion that the aneurysm in my specimen is due not to endocarditis but to a weakness in this region on account of the misplaced septum of the ventricle.

In the normal heart the septum of the ventricle marks the left border of the aorta as may easily be observed by inspecting the cavity of the left ventricle through the aorta. This is also easily demonstrated by 'coronal' sections through the heart as

[1] Rokitansky, Die Defecte d. Scheidewand des Herzens, Wien, 1875.
[2] von Buhl, Zeitschrift f. Biologie, Bd. 16, 1880.

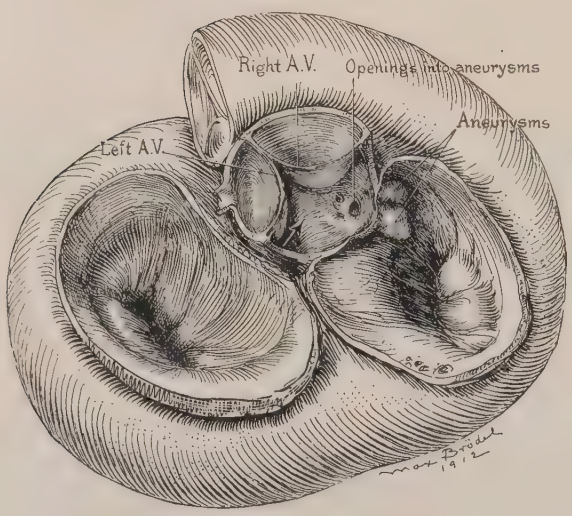

Fig. 1 Base of the heart. Three-fifths natural size. The posterior semilunar valve has been removed from the aorta to show the aneurysm. The right and left aortic (semilunar) are marked.

may also be seen in figures given by Quain, Toldt and Spalteholz. Often, however, the fleshy inferior septum protrudes a little and in such specimens the right semilunar valve (BNA) is attached to it as is normally the case in the ox and the pig. In the specimen under discussion the semilunar valve does not reach to the fleshy septum (fig. 2), the membranous septum extends, not upward, but toward the right, to reach the right side of the root of the aorta. In so doing this portion of the heart wall is weakened, and this may account for the bulging of the membranous septum first into the medial cusp of the tricuspid valve and thence into the atrium. Subsequently this aneurysm might rupture into the right atrium to produce a direct communication between the left ventricle and right atrium as described by Rokitansky and by von Buhl.

At the attachment of the aorta to the heart there is the well known space, which does not empty itself in systole, the space into which the semilunar valves project in diastole according to Henle, called the 'vestibule' in Quain's Anatomy, but not

especially designated in the BNA. This space is relatively much larger in the embryo especially while the interventricular foramen is still present. In studying the development of this region I found it convenient to retain the name 'vestibule' for the space in the ventricle from which the aorta rises. This point is brought out in some detail and illustrated in a study on the development of the heart which will be published shortly in The American Journal of Anatomy.

The aorta in its development must shift from the right side of the heart to the left and after it has gained its permanent

Fig. 2 Section through the heart showing the attachment of the aorta and the aneurysm. Three-fifths natural size.

position the inferior septum blends with its right side, forms the membranous septum, and completes the wall between the left ventricle (vestibule) and the right ventricle. In so doing the inferior septum must shift from left to right while the aorta is shifting from right to left. When this shifting does not complete itself the aorta remains in free communication with the right ventricle, or arises from it, and the interventricular foramen remains open. In such cases the vestibule of the adult remains identical with that of the embryo.

It is generally believed that the septum of the ventricle grows upward from the apex of the heart to unite finally with the

septum intermedium. This method of expression has been challenged by Flack[3] who asserts that the inferior septum is formed by the downgrowth of the two ventricles whose adjacent walls unite secondarily. Finally the cleft makes the notch between the right and left ventricles at the apex of the heart. This view I am able to corroborate. In the heart of an embryo 2 mm. long (no. 391) the canal connecting the two ventricles (ventricle and bulb) is 0.1 mm. in diameter. In an embryo 3.5 mm. long no. 164) it is still but 0.1 mm. in diameter, but in one 3.9 mm. long (no. 463) it is 0.15 mm. in diameter and within it there is a small ridge of tissue, the beginning of the septum inferior. In a specimen 8 mm. long (no. 113) the ventricles are well formed and the interventricular foramen is 0.3 mm. in diameter. At 11 mm. (no. 353) the foramen has reached its maximum size, being 0.4 mm. in diameter, but the septum inferior has extended far down with the growth of the ventricles and there is a cleft apex as is so frequently seen in embryonic hearts.[4] So at first the upper border of the septum grows away from the base of the heart and not towards it. The upper portion of the septum appears first and is therefore its oldest part; in its subsequent development the septum becomes larger by the downward growth of the ventricle. Later the enlarged interventricular septum is closed by the union of the inferior septum with the left wing of the septum intermedium, thus forming the membranous septum just below the medial cusp of the tricuspid valve.

It is apparent from this description that the interventricular foramen is at first gradually enlarging while the aorta is shifting from the right to the left side of the heart. At the same time the right side of the septum intermedium is projecting into this opening, for from it the medial cusp of the tricuspid valve arises. Ultimately this portion of the septum intermedium comes to ride on the posterior part of the septum inferior with the atrio-ventricular bundle between them. This leaves the aorta in front and to the left of the medial cusp of the tricuspid valve with

[3] Flack, In the Further advances in physiology, Longmans, New York, 1909.
[4] Mall, Anatomical Record, vol. 6, 1912.

the communication from the vestibule of the aorta to the right ventricle under the cusp exactly along the line of the right limb of the atrio-ventricular bundle in the adult. The left half of the septum intermedium remains free and is transformed directly into the anterior cusp of the mitral valve. By a subsequent shifting the posterior semilunar valve of the aorta becomes attached to the septum intermedium and does not remain upon the inferior septum as is the case in the heart pictured in fig. 1.

The membranous septum should, therefore, lie just below the anterior end of the medial cusp of the tricuspid valve as illustrated in the atlases of Spalteholz and Toldt and not above it, as stated by Quain.[5] In fact its usual portion is as shown in Spalteholz and Toldt but in exceptional cases the valve becomes adherent to the membranous septum and therefore it appears to be above the base of the valve, that is, between the vestibule of the aorta and the right atrium.

In the specimen under consideration the septum inferior is too far to the left, that is, it should have shifted more to the right. When viewed through the aorta (fig. 1) the septum covers half of its origin and the membranous septum is not in a perpendicular but in a horizontal direction (fig. 2). The horizontal septum naturally becomes the weak portion of the heart, and this may account for the numerous holes through it communicating with the sacs within the medial cusp of the tricuspid valve, as well as, bulging directly into the right atrium. The membranous septum is cribriform. The hypertrophy of the muscle of the heart may be due to the general muscular development of the cadaver from which this specimen came and not to the aneurysm. In the course of time the vigorous heart would favor the rupture of the aneurysm into the right atrium.

Merkel[6] describes a case in which together with an interventricular opening there was an aneurysm from the left ventricle

[5] Quain, vol 2, 1892, erroneously marks with a star the membranous septum in fig. 310. What he has thus marked is really the septum intermedium connecting the mitral and tricuspid valves. The membranous septum lies anterior to this star and is therefore not to be seen in this figure. The text on p. 365 is also incorrect.

[6] Merkel, Virchow's Arch., vol. 48, p. 488.

into the medial cusp of the tricuspid valve. Zahn[7] describes a similar case with multilocular aneurysm into the same valve. In this case there was a displaced fleshy septum as in my specimen. Zahn gives a second case with the inferior septum projecting into the vestibule of the aorta. In this there were several aneurysms from the vestibule through the membranous septum into the tricuspid valve, one of which protrudes into the right atrium, and another projects deep into the septum of the ventricle, as is also the case in fig. 2. In neither of Zahn's cases was any endocarditis present. Rokitansky gives a similar case (fig. 46) with marked protrusion of the ventricular muscular septum into the vestibule of the aorta. This heart was enlarged and there was no endocarditis. In this case there were numerous communications between the left ventricle and the right atrium. Von Buhl describes several cases of aneurysm and rupture of the membranous septum with acute endocarditis. One of his cases (fig. 16) of long duration, had a large communication between the left ventricle and right atrium, together with an opening into the right ventricle, as well as a small unruptured pocket. There was cicatricial thickening around the openings. It is impossible to determine from von Buhl's description whether or not the inferior septum projects into the vestibule of the aorta as in the other case of this catagory. However, von Buhl is of the opinion that his case is due to endocarditis, although he admits that the endocardial thickening may be of secondary origin.

MacCallum[8] describes a similar specimen. One of his specimens which I have had the opportunity to study (fig. 3), shows a complete hollowing of the anterior tip of the medial cusp of the tricuspid valve with a projection below the valve behind this. The distended valve projects into the right atrium and the muscular inferior septum is immediately below the opening of the aorta. The valves are smooth and transparent, no indication of endocarditis being present. The degree of distention of the valve is in advance of that in my specimen. Here again

[7] Zahn, Virchow's Arch., vol. 72, p. 206.

[8] MacCallum, Johns Hopkins Hospital Bulletin, no. 108, March, 1900.

the inferior septum is displaced to the left, thus weakening the membranous septum which must have been placed in a horizontal position.

In my own case the mitral and tricuspid valves were somewhat thickened but not sufficiently to warrant calling the condition due to endocarditis. Certainly there was no acute endocarditis present. However, from all that I can ascertain this type of anomaly can properly be traced back to an embryonic arrest of development in which the inferior septum did not move to the right sufficiently far but remained within the vestibule of the aorta as is the case normally in the ox and the pig. Most of

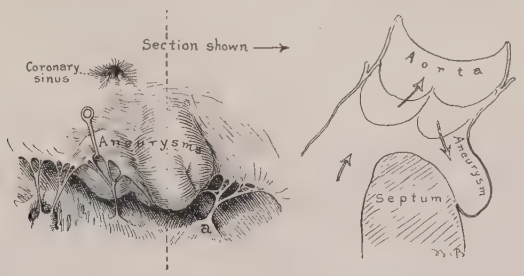

Fig. 3 MacCallum's specimen. One-half natural size. The protrusion of the aneurysm into and below the medial cusp of the tricuspid valve and a diagrammatic section through it are shown.

the cases enumerated above had in them the same anamolous position of the inferior septum. As a result of the misplaced inferior septum the membranous septum develops improperly and becomes placed in a horizontal and not in a perpendicular position. So it is weakened in every way and this predisposes to the formation of aneurysms. Normally the membranous septum lies below the tricuspid valve but a slight distortion may place the origin of the medial cusp of the tricuspid valve upon the membranous septum and as aneurysm from it would naturally invade this valve. The point of union of the medial and anterior cusps anteriorly marks the end of the septum aorticum posteriorly, so any projection over this line extends into the right atrium, a direction which is naturally favored on account of the low pressure in the atria when the ventricles contract.

The conclusion is that aneurysms of this variety are due not to endocarditis but to an anomalous position of the aorta which misplaces the membranous septum into a horizontal position, In both specimens studied, as well as those described in the literature, the inferior septum is displaced to the left and the aorta to the right.

BOOKS RECEIVED

TEXT BOOK OF MICROSCOPIC ANATOMY, Edward Albert Schäfer, 1001 illustrations and 21 colored plates, 739 pages including index, 1912, $7.50, Longmans, Green & Co., New York.

Extract from Preface: The present part of Quain's Anatomy is intended to serve as a Text-Book of Microscopic Anatomy. With this view, the subject-matter has been re-arranged and re-written, and a large number of new figures added. Many are original, the rest are from various sources.

The chapter dealing with the structure of the Vascular System is by Professor G. Mann of Tulane University, New Orleans.

The Index has been prepared by Dr. John Tait of Edinburgh University, who has also assisted in reading the proofs.

THE CONNECTION OF THE VOMERONASAL NERVES WITH THE ACCESSORY OLFACTORY BULB IN THE OPOSSUM AND OTHER MAMMALS

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SEVEN FIGURES

It is well known that there exists in most mammals an accessory olfactory bulb, which is similar in structure and in its peripheral and central connections to the chief olfactory bulb, though it is much smaller and is distinctly separated from it. On the other hand, it has been clearly shown that the vomeronasal or Jacobson's organ is olfactory in character and that it gives origin to nerve filaments, like those of the olfactory mucosa, which form bundles that pass upward through the cribriform plate to the region of the median surface of the olfactory bulb. It has not, however, been recognized, as far as the writer knows, that the fibers arising from the vomeronasal organ and the fibers connected with the accessory bulb are one and the same thing. It is the purpose of the present paper to report some observations which establish this connection, and which show that we have in the vomeronasal nerves the combined filaments from the vomeronasal organ, and that they are the fibers that terminate in the accessory olfactory bulb and are the only fibers it receives. We thus have in the accessory olfactory bulb a receptive station for the stimuli coming from the mucosa of the vomeronasal organ.

The writer's attention was turned toward this subject in the course of an investigation of the olfactory apparatus of the

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opossum which he has undertaken as a part of a general study of the central nervous system of this animal that is being carried on in this laboratory. In tracing the origin and distribution of the olfactory nerve filaments it gradually became apparent that a special group of fibers could be isolated that arose in the vomeronasal organ and terminated in the accessory bulb. A subsequent examination of other mammals showed that a similar condition existed in the rodent group, the ungulates, the cat and the dog. In all of them the vomeronasal nerve filaments exhibit a characteristic difference from the olfactory filaments both in their course, size and termination. Before reporting my own observations in detail I will briefly review the literature concerning this region so far as it has come to my notice.

Apparently from the first it was supposed that Jacobson's or the vomeronasal organ was olfactory in character. It was early shown by Balogh ('60), Klein ('81 a) and Piana ('82) that its mucosa contained neuroepithelial cells like those found in the olfactory mucosa. Subsequently v. Brunn ('92) and Lenhossék ('92) demonstrated that these cells gave origin to axones that passed upward in the nasal submucosa of the septum like the olfactory nerve filaments. Read ('08) confirmed these observations and carefully traced the course of these fibers from Jacobson's organ through the cribriform plate to the region of the median surface of the olfactory bulb. Following the terminology of DeVries ('05) she used the words ' nervus vomeronasalis' as including the whole group, thereby distinguishing them from the ordinary olfactory fibers.

The existence of the accessory olfactory bulb in mammals was first recognized by v. Gudden ('70). He described it in the rabbit as a slightly elevated oval body, lying on the olfactory peduncle posterior to the olfactory bulb, which in structure resembles the olfactory bulb, like the latter receiving olfactory filaments and giving off fibers to the lateral olfactory tract. Ganser ('82) reports an accessory olfactory bulb in the mole. He describes it as smaller than that in the rabbit, but as in the rabbit it is similar in structure and lamination to the olfactory

bulb. Koelliker ('96) was the first writer to illustrate the structure and relations of the accessory olfactory bulb. In his Gewebelehre he shows a transverse Wiegert stained section through the olfactory bulb of the rabbit which clearly shows the relation between the accessory olfactory bulb and the main olfactory formation. Ziehen ('97) mentions a structure in marsupials (Pseudochirus) similar to that described by Gudden in the rabbit, wedged in between the olfactory bulb and olfactory peduncle at the dorsomedian angle. Cajal ('11) describes the accessory olfactory bulb of the mouse, rabbit and guinea pig as a circular structure situated on the superior surface of the olfactory bulb. It is hemispherical in shape and in cross section semilunar. A special bundle of olfactory fibers reaches it by coursing transversely and terminates by loose arborizations within the glomeruli. According to his description there are no true mitral cells present but in their places he describes triangular and stellate cells of small and medium size whose dendrites pass outward and terminate in loose arborizations. The axis cylinders pass to the thin layer of fibers that lie beneath and go to the lateral olfactory tract. A layer of granules lies below the latter and resembles the granules of the olfactory bulb but are much smaller.

There are three important papers to which special attention should be directed as they directly cover the work with which we are involved. The first of these is by Balogh ('60) whose valuable contribution has been unduly overlooked by most writers. He clearly traced in the sheep filaments arising from the median surface of Jacobson's organ which pass upward to the cribriform plate in three or four well defined bundles. After entering the cranium by a single foramen the single trunk thus formed turns lateralward to terminate in 'Jacobson's hill.' Judging from the figures (12 to 14) accompanying his paper the structure he named as 'Jacobson's hill' is plainly what we now know to be the accessory olfactory bulb. He recognized and successfully traced the fibers from Jacobson's organ to their termination, but the histological character and the central connections of his Jacobson's hill were of course unknown to him.

In 1897 G. Elliot Smith described the olfactory bulb of a foetal ornithorhynchus. He speaks of it as imperfectly covered by two distinct ganglionic masses. The larger median mass covered the median surface and extended onto the dorsal and ventral surfaces. It received the terminations of the olfactory nerves. He called this the 'olfactory' ganglion. On the lateral aspect of the bulb is the smaller mass which receives the terminations of the nerves arising from Jacobson's organ. This mass he termed the 'ganglion of Jacobson's organ.' It seems very probable that his ganglion of Jacobson's organ was the same thing as Balogh's Jacobson's hill and that he had before him the embryonic accessory olfactory bulb.

The third paper is by Döllkin ('09) who studied Cajal preparations of very young embryos of man, rabbit and mouse. DeVries ('05) had previously studied the human embryo and had concluded that the vomeronasal nerves were the same thing as the nervus terminalis found in lower forms. Döllkin's material was more abundant than DeVries' and his results more definite. He successfully traced the nerves from the vomeronasal organ to a ganglionic mass on the oral extremity of the hemisphere which he termed the 'ganglion terminale.' As the stages with which he worked were before the formation of the olfactory bulb, he did not clearly determine the relation of the ganglion terminale to the olfactory bulb on the one hand or the preterminal area on the other. He concluded, however, that it was connected with the latter area and agreed with DeVries in believing that the vomeronasal nerve and the nervus terminalis were the same thing. He shows ganglion cells scattered along the course of the vomeronasal fibers.

The question of the nervus terminalis and its relation to the vomeronasal nerves I shall not take up in the present paper as my studies have been restricted to mammalian forms which are not adapted to that problem. Important work in that direction has already been published (Herrick, '09; Sheldon, '09; Brookover, '10; Brookover and Jackson, '11; McKibben, '11).

MATERIAL AND METHODS

The observations about to be reported were based in part on serial sections, stained by the Wiegert method, of the opossum and the rat, and in part on dissections of fresh and prepared specimens of one or more of the following species: opossum, bat, rat, rabbit, guinea pig, sheep, cat, dog and reptiles (snake and gila monster). Figs. 2 to 7 represent drawings made from such dissections. In both the rat and the opossum wax plate reconstructions were made to show the detail in shape and relative size of the accessory olfactory bulb.

For purposes of dissection it was found advantageous to immerse the specimen, after it had been divided just to one side of the median sagittal plane, in Müller's fluid to which had been added 5 per cent glacial acetic acid. After the specimen had been in this fluid from twelve to twenty four hours its component nerve fibers were found toughened and differentiated in color facilitating their identification. The dissection was done under water or weak alcohol with a binocular microscope. The first step in the procedure consisted in the removal of the nasal septum, thereby exposing the vomeronasal organ and the nerves arising from it. It is then easy to trace the nerves upward in the submucosa to where they terminate in the accessory olfactory bulb.

PERSONAL OBSERVATIONS

Concerning the microscopical appearances of the accessory olfactory bulb I have very little to add to the descriptions already given by Kölliker ('96) and Cajal ('11). In fig. 1, however, I am able to show the appearance as found in the opossum which constitutes a very favorable species for the study of this structure. This figure represents a transverse section through the caudal part of the olfactory bulb stained by the Wiegert method. The accessory olfactory bulb can be seen in the dorsal portion constituting a little more than half of the thickness of the brain wall. Like the olfactory bulb it consists of four layers: (1) superficial fiber layer, (2) layer of glomeruli, (3) layer of gray substance or molecular layer and (4) the granular layer. The

vomeronasal nerve can be seen at the median border where it spreads out to form the superficial fiber layer. The glomeruli are smaller than those of the olfactory bulb and appear to be grouped in smaller and more irregular masses. The molecular layer differs in that the cells corresponding to the mitral cells

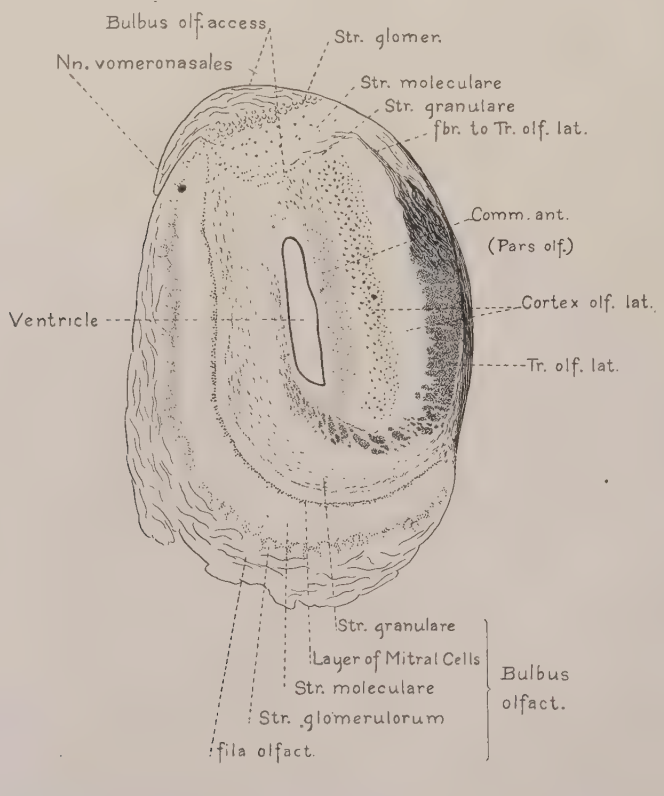


Fig. 1 A transverse section through the caudal part of the olfactory bulb of the opossum illustrating its relation to the accessory olfactory bulb and the termination of the vomeronasal nerves. From a Wiegert preparation (our series, Op. A., slide 8, section 7). Magnified ten diameters.

are not arranged along a sharply marked line as in the olfactory bulb but are irregularly placed throughout the whole layer. The granular layer forms the innermost layer of the accessory olfactory bulb and projects with a convex surface

toward the remainder of the bulb. Its constituent cells are clumped in characteristic islands from between which fibers can be seen emerging which pass lateralward to join the main lateral olfactory tract.

It will be noticed that the term olfactory bulb' is used in the sense as used by Kölliker, that is to include only: (1) stratum glomerulorum (with its fila olfactoria); (2) stratum moleculare (including the mitral cells); and (3), stratum granulare. 'Olfactory formation' is a term used by some writers for the same thing. It refers only to the cap-like mass covering in the olfactory evagination and does not include necessarily the fiber bundles coming to and going away from it. Thus in fig 1 the olfactory bulb forms only the ventral and median part of the section. The accessory bulb forms the dorsal part of the section, while the lateral half of the section is formed by a strand of fibers from the anterior commissure, by the bundles of fibers constituting the lateral olfactory tract and between these two a forward extention of the lateral olfactory cortex belonging to the pyriform lobe.

In describing the appearances of these structures as seen in the dissections each animal will be taken up separately. In most cases only one dissection was made and no attempt was made to determine the amount of variation. Judging, however, from the comparison of the different ones it is evident that the variation is not great.

Opossum

The olfactory bulb of the opossum (fig. 2) extends horizontally forward beyond the hemisphere. It is ovoid in shape with a well defined oblique groove determining its junction with the short stout peduncle. The median surface is flattened against the bulb of the opposite side. The ventral part of this surface extends one-third of its length farther caudally than the lateral. The superior and lateral surfaces are comparatively smooth and convex. The anteroventral surface lies upon the concave dorsal aspect of the cribriform plate and receives the numerous large olfactory nerve filaments which spread out in a network

of varying thickness over the entire surface of the olfactory bulb.

The accessory olfactory bulb presents an oval elevated surface lying on the dorsomedian aspect of the olfactory peduncle at the caudal margin of the olfactory bulb. It is separated from the olfactory bulb and peduncle by a shallow groove. The blunt rounded extremity of the frontal lobe of the cerebrum covers its caudal border.

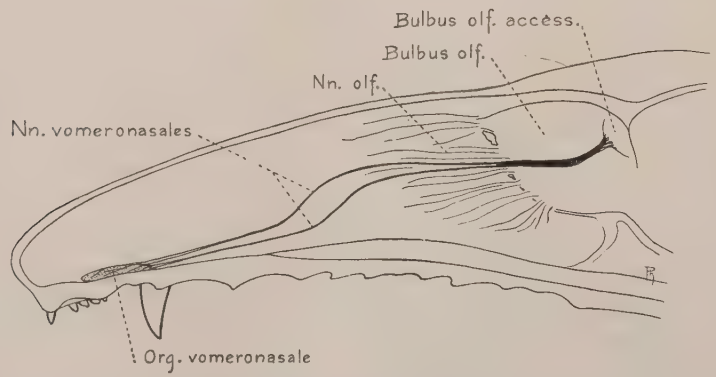


Fig. 2 Median section of the head of an opossum with the mandible and nasal septum removed to show the course and connections of the vomeronasal nerves. Natural size.

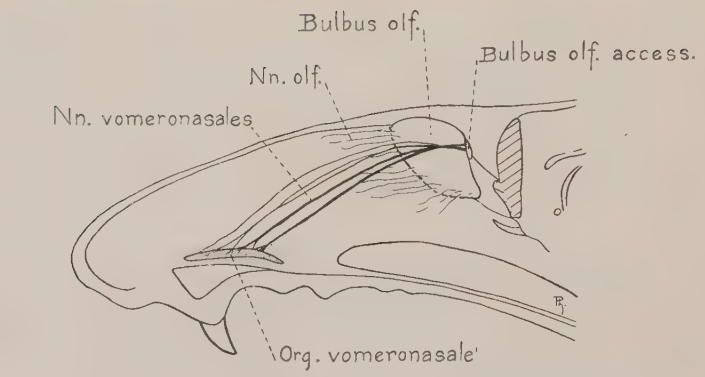
The vomeronasal nerves arise as a large number of fine filaments coursing obliquely upward and backward on the median wall of the vomeronasal organ. Immediately dorsal to the organ they unite to form two well defined nerves which course parallel to one another and horizontally backward in the submucosa of the much elongated septum to the cribriform plate. These nerves pass directly to the cribriform plate except for a short double curve in the region of the junction of the septal cartilage with the perpendicular plate of the ethnoid bone. After entering the cranium by separate foramina, accompanied by olfactory filaments, the two nerves unite and pass upward and backward over the median surface of the olfactory bulb to the anteromedian border of the accessory bulb where they spread out in a network over its surface.

Rat

In its outer form the olfactory bulb of the rat (fig. 3) resembles that of the opossum. Its long axis, however, extends obliquely upward and forward instead of horizontally forward as in the opossum and as will be seen in the rabbit. It is partly covered in above and posteriorly by the frontal lobe of the cerebrum. The median surface is flattened against the opposite olfactory bulb, the dorsal and lateral surfaces are convex. The antero-ventral surface lies upon the obliquely placed lamina cribrosa. The posterior border of the olfactory bulb is determined by an irregular oblique groove which sharply marks its junction with the olfactory peduncle.

The accessory olfactory bulb forms a small oval mass occupying a shallow fossa on the dorsal surface of the olfactory peduncle. It is slightly raised above the smooth surface of the peduncle and separated from it by a shallow groove. It is entirely hidden from view by the forward extention of the frontal lobe of the cerebrum, and in order to see it, it is necessary to remove a portion of the cerebrum as is shown in fig. 3.

The vomeronasal nerves begin as a group of small filaments which arise from the ventral and median walls of the vomeronasal organ. These filaments curve around the median surface of the organ and leave its dorsal margin in four well defined nerve bundles. The more caudal two are somewhat larger, while the more oral two are more slender and show some tendency to plexus formation. These nerve bundles course dorsocaudally in the submucosa on the surface of the septum until they reach the lamina cribrosa. Here they enter the cranium by separate foramina accompanied by some of the olfactory filaments. After entering the cranium they unite on the median surface of the olfactory bulb into one nerve trunk which courses caudally to the caudal border of the bulb where it turns lateralwards to the accessory olfactory bulb. The nerve spreads out over the surface of the accessory bulb to form its superficial fiber layer.



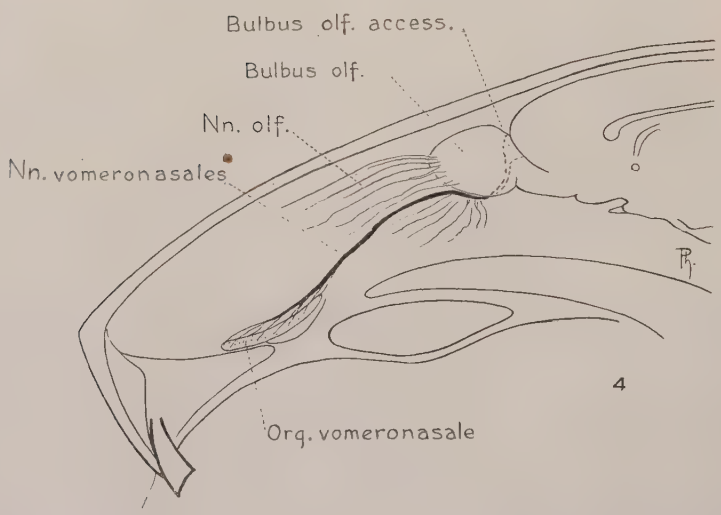


Fig. 3 A median section of the head of a rat with the mandible, the nasal septum and the anterior portion of the cerebrum removed to show the origin, course and termination of the vomeronasal nerves. Magnified two diameters.

Fig. 4 Median section of the head of a guinea pig showing the origin, course and termination of the vomeronasal nerves. The nasal septum and mandible have been removed. Magnified two diameters.

Guinea pig

The olfactory bulb of the guinea pig (fig. 4) resembles that of the opossum and the rabbit in extending directly forward so that the longest axis corresponds to the longitudinal axis of the brain. It thus happens that it is only partly covered in by the frontal lobe of the brain. The median surface of the bulb is the largest and flattened against the bulb of the other side. It extends about one third of its length farther caudally than the lateral surface. The anteroventral surface receives the olfactory filaments from the olfactory mucosa and lies upon the obliquely placed lamina cribrosa. The olfactory bulb is connected with the main mass of the brain by a short slender peduncle.

The accessory olfactory bulb forms a small white hemispherical swelling on the dorsolateral aspect of the olfactory peduncle at the caudal margin of the olfactory bulb. It is circumscribed by a shallow groove which makes a sharp boundary between it and the olfactory bulb and peduncle. The forward extention of the frontal lobe entirely obscures it from view when looked at directly from above. It may be clearly seen, however, in a lateral view of the brain.

As in the opossum and the rat the vomeronasal nerves arise by numerous filaments from the median surface of the vomeronasal organ. These filaments extend obliquely upward on the median surface of the organ and become assembled into a single trunk along its dorsal margin. From the posterior extremity of the organ the vomeronasal nerves continue as a single trunk dorsocaudally through the submucosa of the nasal septum to the lamina cribrosa through which they pass by a single foramen into the cranial cavity. Its course within the cranial cavity is different from that of all other mammals examined. Instead of passing upward on the median surface of the olfactory bulb, it takes a curved course on the upper surface of the cribriform plate around behind the more caudal olfactory filaments to reach the lateral surface of the olfactory bulb. Thus in the guinea pig the vomeronasal nerves, instead of reaching the acces-

sory bulb by a median course, pass spirally under the olfactory bulb and enter the accessory bulb at its ventral and lateral borders.

Rabbit

In the rabbit (fig. 5) the olfactory bulb extends forward beyond the hemisphere. It is oval, compressed from side to side and its long axis lies in the horizontal plane. The posterior border is determined by an irregular, oblique, circular groove which indicates its union with the olfactory peduncle. The antero-ventral surface receives the olfactory nerve filaments which spread out over the surface of the bulb in a thick network of

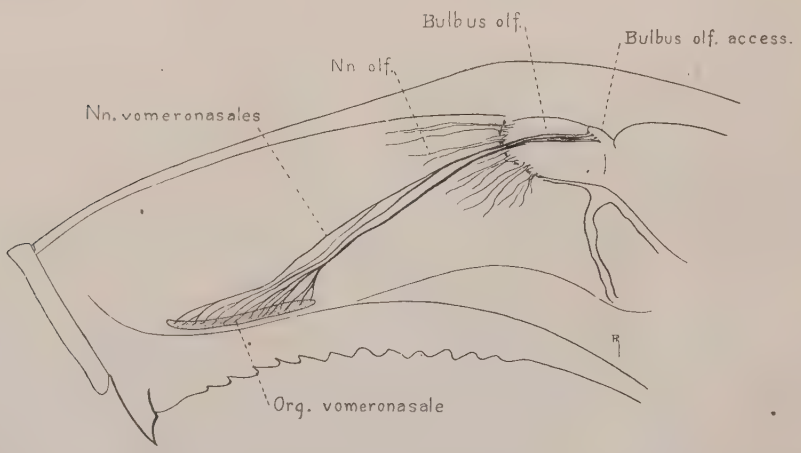


Fig. 5 A median section of the head of a rabbit showing the origin, course and termination of the vomeronasal nerves. The nasal septum and mandible have been removed. Natural size.

fibers. The median surface is flattened against the opposite olfactory bulb. It extends one-third of its length farther caudally than the lateral surface. The lateral and dorsal surfaces are convex and appear smoother than the others.

On removing the dorsal part of the cranium and meninges there can plainly be seen the accessory olfactory bulb lying between the caudal border of the olfactory bulb and the anterior rounded extremity of the frontal lobe of the cerebrum. It is

a small structure producing an oval eminence on the dorsomedian aspect of the olfactory peduncle. The olfactory bulb is separated from it by an interveing portion of the olfactory peduncle.

The vomeronasal nerves arise by numerous fine filaments from the ventral parts of the median urface of the vomeronasal organ. From their origin they pass obliquely upward over this surface being joined by other fibers in their course. Along the dorsal margin of the organ they unite with one another to form about eight well defined nerve bundles. Here the two nerve bundles formed by the union of all the filaments from the posterior half of the organ unite into one large bundle which courses upwards and backwards through the submucosa of the septum to the lamina cribrosa. The remaining six nerve bundles which represent the filaments arising from the anterior half of the vomeronasal organ run parallel close to one another, and at about two thirds of the distance to the lamina cribrosa they unite into one trunk which continues its course to the cribriform plate. After passing through the same foramen the two nerve bundles unite and pass posterior on the median surface of the olfactory bulb. At about the middle of their course over the bulb the nerve divides into three nearly equal parts which continue an individual course to the posterior border of the bulb where they unite again and turn laterally to the anterior margin of the accessory bulb. Here the nerve spreads out to cover its surface with a plexiform layer of fibers. Thus in the rabbit the bundles forming the vomeronasal nerves are united in a single trunk only in one part of their course, and that is where they pass through the foramen in the cribriform plate. In the other regions they are grouped into between three and eight separate bundles. As in most of the animals examined the more caudal bundles have a tendency to be larger then the others.

Sheep

The olfactory bulb of the sheep (fig. 6) lies in a deep fossa on the cribriform plate so that it is hidden from view in a median sagittal section of the head. From above it is pressed forward

by the frontal lobe of the brain which fits closely in its concave dorsocaudal surface (see dorsal view, fig. 6). The cribriform plate approaches a vertical position and the axis of the olfactory bulb forms an angle of about 130 degrees with the peduncle. Thus the ventral surface in which the olfactory nerves terminate is crowded upward and presents a convex surface that is perpendicular to the olfactory mucosal regions.

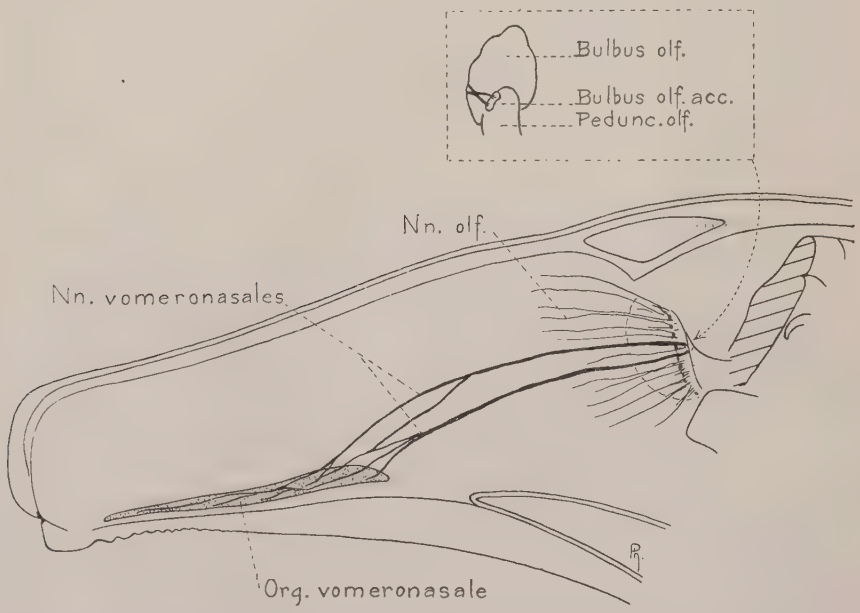


Fig. 6 Median section of the head of a sheep with the frontal lobe of the brain, the nasal septum and the mandible removed showing the origin and course of the vomeronasal nerves. A dorsal view of the olfactory bulb is shown above indicating the termination of the nerves. Two-thirds natural size.

The size and position of the accessory olfactory bulb is indicated in fig. 6. It lies on the dorsal surface of the olfactory peduncle at the caudal margin of the olfactory bulb and is completely under cover of the frontal lobe of the brain. It forms an oval mass about twice as long as broad and is outlined by a shallow groove. Its posterior margin has an indentation which partly separates it into two lobes.

The vomeronasal nerves arise by several small filaments from the anterior extremity of the large elongated vomeronasal organ. These filaments course in an oblique logitudinal direction on the median surface of the organ to its posterior extremity, increasing in size by the addition of other filaments. Here they leave as four well defined nerve trunks. In the first half of their course to the lamina cribrosa the nerves join one another in a plexiform manner and finally form two large nerve trunks which course to the cribriform plate. These two nerve trunks pass through separate foramina accompanied by olfactory nerve filaments. After entering the cranium they bend sharply around the dorsal margin of the olfactory bulb to reach its posterosuperior surface. Here they unite into one nerve which almost immediately divides again into two short, stout trunks, one going to each extremity of the accessory olfactory bulb to spread out over its surface.

Cat

Viewed from the median aspect the olfactory bulb of the cat has a quadrilateral outline with a long axis passing from above caudoventralward. It is compressed from side to side. The lateral and median surfaces are large. The former lies against the concave bony wall of the brain case. The latter is flat and lies in apposition to the median surface of the olfactory bulb of the opposite side. The dorsal, caudal, ventral and oral surfaces are narrowed at the expense of the lateral and median. The dorsal surface is convex smooth and lies in contact with the anterior part of the vault of the cranium. The caudal surface is covered by the forward extention of the frontal lobe of the brain. From the ventral part of this surface the olfactory peduncle extends caudally to the main mass of the cerebrum. The convex ventral surface lies upon the floor of the anterior cranial fossa. The anterior surface lies against the obliquely placed lamina cribrosa and receives the olfactory nerves which spread out over the surface of the olfactory bulb.

The accessory olfactory bulb in the cat is about the same size as in the opossum. It is oval slightly elevated from the sur-

rounding surface of the dorsomedian aspect of the olfactory peduncle at the caudal margin of the olfactory bulb.

The vomeronasal nerves arise by numerous filaments from the ventral border of the vomeronasal organ. They course obliquely upward and backward on its median wall and leave the dorsal border as two small trunks. These nerve trunks continue the dorsocaudal course to the lamina cribrosa where they enter the cranium by separate foramina. They unite immediately after entering the cranium. The single nerve trunk thus formed extends upward and backward on the median surface of the olfactory bulb to its caudal border. Here it turns sharply lateralward over the posterior surface to reach the anterior margin of the accessory olfactory bulb.

Dog

The large quadrilateral shaped olfactory bulb of the dog (fig. 7) is compressed into its particular shape by the frontal pole of the cerebrum on one side and the framework of the nasal passages on the other. The dorsocaudal surface is concave and moulded to the anterior extremity of the frontal lobe. The olfactory peduncle extends backward from the ventrolateral part of this surface. The anteroventral surface lies against the obliquely placed cribriform plate. It receives the olfactory nerves which spread out in a thick network over the surface of the olfactory bulb. The median thickened border of the olfactory bulb is separated from the opposite bulb by the dorsal extension of the perpendicular plate.

The accessory olfactory bulb is smaller in the dog than in the rabbit, rat and guinea pig. It forms a small oval elevation on the median surface of the olfactory peduncle at the caudal margin of the olfactory bulb. It may be seen only upon careful removal of the pia mater.

The vomeronasal nerves arise by a large number of filaments from the median surface of the vomeronasal organ. These filaments unite in such a manner that eight nerve strands leave the dorsal border of the organ. Soon after leaving they unite to form four large bundles. The four bundles turn caudally

and course through the submucosa of the nasal septum to the cribriform plate. They enter the cranium by two openings and then unite into a single nerve trunk, which passes around the median thickened border of the olfactory bulb, where, turning lateralward on its dorsal surface the nerve separates out in a network of fibers which terminates in the accessory olfactory bulb. On the dorsal surface of the olfactory bulb the vomeronasal nerves are closely applied to the layer of fila olfactoria.

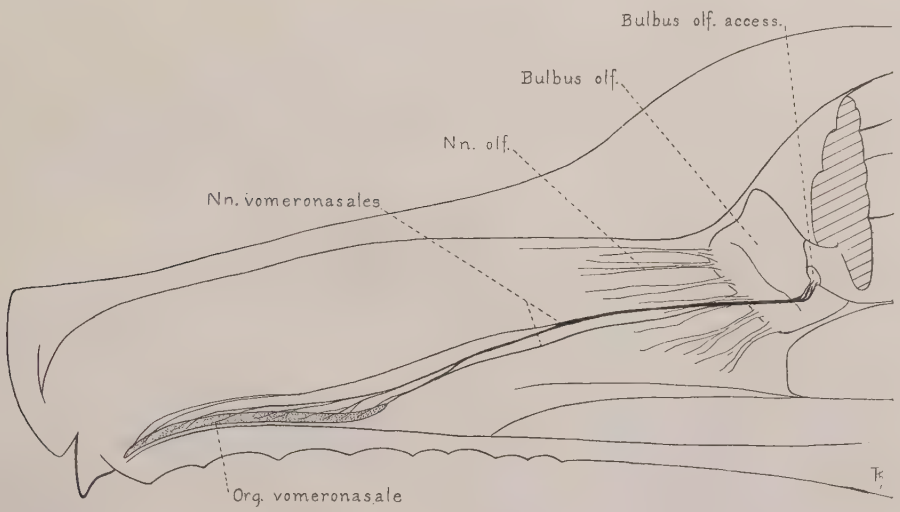


Fig. 7 A median section of the head of a dog with the frontal lobe of the brain, the nasal septum and mandible removed showing the origin, course and termination of the vomeronasal nerves. Natural size.

It may be mentioned that what seemed to be a small nerve bundle left the group of vomeronasal fibers near the accessory olfactory bulb and passed caudalward to the median surface of the brain and terminated in the ventral part of the precommissural area. This would suggest the nervus terminalis of Pinkus. This, however, was only found in one specimen and nothing definite can be said of it until it is more carefully worked out.

Reptiles

It is beyond the purpose of the present paper to trace the changes in the vomeronasal nerves in submammalian forms. However, dissections were made of the snake and gila monster (heloderma suspectum). In the former the vomeronasal nerves form two large bundles which pass backward on the median surface of the olfactory evagination and finally unite and then terminate in a ganglion mass situated on the dorsal and median surface of the evagination, which is nearly as large as the olfactory formation itself. This corresponds closely to the conditions described by Herrick ('93). In the gila monster the vomeronasal nerves terminate in a similar ganglion mass though it is not so large. Evidently these ganglion masses corresponds to the accessory olfactory bulb of mammals.

SUMMARY

It will be seen from the foregoing descriptions of the accessory olfactory bulb that it is a ganglionic mass for the reception of fibers from the vomeronasal organ of Jacobson and that centrally it gives off fibers that join the lateral olfactory tract. It is entirely separate from the olfactory bulb, though it is contiguous to it, being always situated on its dorsocaudal surface. Its size varies directly with the size of the vomeronasal organ.

It is evident that Balogh's 'Jacobson's hill', Smith's 'Ganglion of Jacobson's organ', Döllkin's 'Ganglion terminale', and probably also Herricks's 'median ganglion mass in reptiles' are the same thing as the accessory olfactory bulb.

The olfactory nerve filaments may be divided into two distinct groups; (a) ordinary olfactory fibers, terminating in the olfactory bulb; and (b) vomeronasal fibers, terminating in the accessory olfactory bulb. It is probable that a third group may be added to include the nervus terminalis, the description of whose central connections are entirely different from either of the first two groups.

Throughout the text of this paper an effort has been made to conform to the nomenclature that is at present in most general

use. I would suggest, however, the substitution of the term 'tuberculum vomeronasale' in the place of 'bulbus olfactorius accessorius'. We would then have the vomeronasal organ, the vomeronasal nerve filaments and the vomeronasal tubercle as the component parts of this apparatus.

In conclusion the writer takes pleasure in acknowledging his indebtedness to Professor Streeter whose constant advice has facilitated the completion of this study.

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A CASE OF ARRESTED DEVELOPMENT OF PANCREAS AND INTESTINE

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ONE FIGURE

The persistence of certain embryonic conditions of the intestine and pancreas, as found in one of the dissecting room subjects, was so rare as to warrant description. The subject was a white female, aged seventy-one who died of 'valvular heart disease'. The body was received two days after death and immediately embalmed with equal parts of alcohol, glycerine, and carbolic acid. This fluid hardens the tissues so as to preclude any alteration in relation of abdominal viscera after embalming. There were no indications of operation on the abdomen before or after death. Furthermore, the attachment of the viscera was such that essential disarrangement would be almost impossible. The peculiarities of the case had to do with the arrangement of the large and small intestine and the form as well as relations of the pancreas.

The stomach was placed vertically in the upper part of the abdomen with the pylorus in the middle line at the level of the pyloric plane; i. e., midway between the suprasternal notch and the symphisis pubis. The cardiac orifice was 10 cm. cephalic to the pylorus in the middle line so that the greater curvature of the stomach was almost entirely in contact with the diaphragm and the left lateral abdominal wall.

The duodenum, lying wholly to the right of the middle line, was of the *V*-shaped variety with the *V* reversed, that is, the ascending limb of the duodenum was to the right rather than to the left of the descending limb. From the pylorus the duodenum

passed cephalically and to the right until it touched the under surface of the gall bladder, turning there and running directly caudally close to the bodies of the lumbar vertebrae. Opposite the fourth lumbar vertebra, at the level of the umbilicus it was bent sharply back upon itself and then passed cephalically at the right of the descending limb and slightly ventral to it.

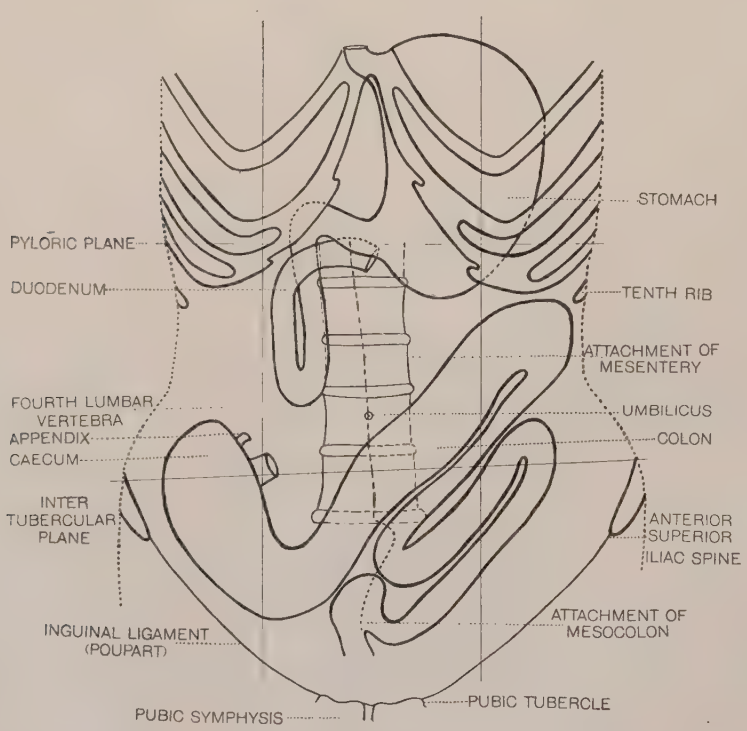

Diagram 1 Outline showing the position in the body of the stomach, duodenum, and colon

At the pyloric plane it turned medially to become continuous with the jejunum.

The remainder of the small intestine was entirely on the right side of the body but presented no further peculiarity.

The caecum was in the right iliac fossa with its tip on a level with the iliac crest and pointing cephalically. The appendix

was quite short, 2.5 cm. in length, but otherwise normal. Its tip pointed cephalically and medially. The ileum entered the caecum about 2.5 cm. from its tip, at right angles to its long axis.

From the right iliac fossa the colon passed caudally and medially into the false pelvis and then turned cephalically to the left passing over the sacral promontory to the tip of the tenth rib on the left side, 9 cm. above the anterior superior spine of the ilium. This part of the large intestine represents what is usually described as the ascending and transverse portions of the colon.

The remainder of the large intestine represents the descending colon, sigmoid flexure and rectum. At the tip of the tenth rib the colon turned sharply upon itself and extended medially and caudally, parallel to the portion just described, to the middle line. At this point, 6 cm. above the symphisis pubis, it turned again to the left parallel and immediately caudal to the first loop. At the level of the iliac tubercle it turned back and passed once more medianward just above Poupart's ligament to the middle line, where it bent first cephalically, then dorsally and finally caudally to become continuous with the rectum.

Thus the small intestine occupied the cephalic and right portion of the abdomen; the large intestine the caudal and left part. In addition both parts were much shorter than is usually found. The small intestine along its mesenteric border measured only 2.05 m. ($6\frac{2}{3}$ feet) and along its free border 2.6 m. ($8\frac{1}{2}$ feet). The large intestine along its free border from the tip of the caecum to the anus, measured 1.17 m. ($3\frac{5}{6}$ feet).

The first, horizontal portion of the duodenum was entirely enclosed in peritoneum directly continuous with that of the stomach. The descending and ascending limbs of the duodenal *V* were enclosed in a single pouch of peritoneum, attached to the dorsal abdominal wall along the lateral surface of the bodies of the vertebrae from the umbilicus to the cephalic end of the line of attachment of the mesentery. The ventral layer of this pouch, after covering the *V* of the duodeunm, extended to the left over the bodies of the vertebrae, and became continuous

with the right layer of the mesentery. This ventral layer of the pouch cephalically covered over, first, that part of the ascending limb of the duodenum which crossed in front of the descending limb to join the jejunum; secondly, the cephalic part of the descending limb; and finally, extended onto the dorsal abdominal wall cephalic to the duodenum. The dorsal layer of the pouch continues with the ventral layer over the lateral surface of the ascending limb of the duodenum, after covering over the dorsal surface of the *V* was continued onto the dorsal abdominal wall. The dorsal and ventral layers of the pouch were in contact part of the way between the two limbs of the *V*.

The mesentery of the rest of the small intestine was attached along a very nearly straight line, beginning above at the pyloric plane 1 cm. to the right of the middle line and ending 15 cm. caudalward immediately to the left of the middle line. Its average length from the intestine to its attachment was 10.75 cm.

The first 15 cm. of the colon, including the caecum, was entirely enclosed in peritoneum which formed for it a mesocolon directly continuous with the mesentery of the small intestine. Where the colon passed over the sacral promontory its mesocolon was lost and its dorsal surface uncovered by peritoneum was attached to the sacrum by areolar tissue. Immediately beyond this point the peritoneum again completely surrounded the intestine forming a mesocolon for it as far as the middle of the sacrum. From this point on the rectum had no mesentery. The average length of this mesocolon from the intestine to its attachment was 7.5 cm. Its root was attached over the ventral surface of the sacrum just to the left of the middle line for a distance of about 4 cm. The point where the colon was without mesocolon corresponded to the caudal limit of the attachment of the mesentery of the small intestine.

The great omentum was not fused with the peritoneal covering of the colon as is usual, but consisted only of two layers of peritoneum, the reflections from the ventral and dorsal surfaces of the stomach. It was lodged in a mass just caudal to the stomach between it and the colon, instead of being spread out as a sheet over the coils of small intestine. It was in con-

tact with the peritoneum of the dorsal abdominal wall over quite an area and was partly fused to it. This adhesion may have been inflammatory.

The rest of the abdominal organs were normal except the pancreas.[1] This consisted of two parts connected by a narrow band of pancreatic tissue dorsal to the duodenum. The smaller portion lay dorsal and to the right of the descending part of the duodenum with its long axis along the common bile duct. It was drained by a small duct opening with the common bile duct on the dorsal wall of the duodenum 6 cm. from the pylorus. The larger portion lay to the left of the descending part of the duodenum partly dorsal to the stomach and extended only 5 cm. to the left. It was drained by a single large duct opening independently 3.5 cm. cephalic and ventral to the opening of the other duct. The narrow band of pancreatic tissue that joined the two portions of the gland was drained by both ducts yet they were not in communication with each other. The entire pancreas was only about the size of the head of an ordinary pancreas.

In this case the rotation of the primitive loop of gut of the sixth week of intrauterine life failed to occur so that the intestine persisted in its primitive relation; i.e., with the small intestine cephalic and to the right, the large intestine caudal and to the left, the only difference being in the increased size of the primitive loop. Also the duodenum instead of rotating to the left turned to the right reversing the relation of the two limbs which in addition affected the relations of the pancreas in that it caused a rotation of the ventral pancreas also to the right instead of to the left. The smaller mass described above would correspond to the ventral, the larger mass to the dorsal pancreas. These two parts fused to some extent as shown by the narrow band of pancreatic tissue, but the anastomosis between the two ducts was not established so that the minor pancreatic duct (duct of Santorini) persisted entire as the larger of the two draining the major portion of the gland.

[1] Described by W. M. Baldwin, Anat. Rec., vol. 4, no. 1, January, 1910.

EXPERIMENTS ON LOCALIZATION AND REGENERATION IN THE EMBRYONIC SHIELD AND GERM RING OF A TELEOST FISH (FUNDULUS HETEROCLITUS)[1]

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SEVENTEEN FIGURES

From experimental studies[2] on a teleost egg (Fundulus heteroclitus) we came to the conclusion that there was no definite localization of organ forming substances in either the germdisc or the blastodisc. Normal but usually smaller embryos develop from the germdisc or blastodisc after the exterpation of various portions of the protoplasm. With the formation of the germ ring and the embryonic shield from the blastodisc a very pronounced change occurs and we find by a very similar method of experimentation that there is a very definite localization and almost complete lack of regenerative power in the embryonic shield. This changed condition appears with the formation of the axis of the embryonic shield, and the contrast with the previous condition is very remarkable. It is evidently associated in some manner with the development of the axial keel or central nervous system. The axial keel when first recognizable under the binocular microscope appears as a highly refractive point in the median line of the early embryonic shield. It elongates with the elongation of the shield, always extending the entire length of the median line of the shield. At or near the posterior end is located the growing point or node of the axis. The extirpation of the entire keel at any stage of the embryonic shield prevents any farther development of the embryo.

[1] These experiments were done at the Marine Biological Laboratory, Woods Hole, Mass.

[2] W. H. Lewis, Experiments on localization in the eggs of a teleost fish (Fundulus heteroclitus). Anat. Rec., vol. 6, no. 1, 1912.

The method used was similar to that employed in the experimenting on the germdisc and blastodisc. The fish were stripped, the eggs fertilized and kept in sea water at room temperature. In experimenting the egg is held by a fine pair of forceps with sufficient pressure to prevent the yolk from turning within the vitelline membrane and then a fine needle is thrust through the vitelline membrane into the embryonic shield at the point desired and as the needle is withdrawn, pressure with the forceps sends out a stream of cells from the injured region leaving a wound, the size and position of which can be readily seen with the binocular microscope. The eggs thus operated upon were kept for days (eight to twenty-four) in small dishes, some hatching, others unable to do so.

Many experiments were done on the embryonic shields of various stages and but a small fraction of them are given in this paper.

Operations on the early embryonic shield

If the central or axial portion of the shield is removed, as shown in figure 1, development stops and the embryo dies. The material in the lateral portions of the embryonic shield and in the germring is apparently incapable of replacing that in and near the median line. At this stage the axial keel makes its first appearance and it is the removal of this structure which determines the result. Unless the axial material is entirely removed, abnormal embryos may result in which various parts are wanting. Sumner[3] did a somewhat similar series of operations with the electric cautery on 96 eggs, 78 died and 10 showed, thirty-three hours afterwards, normal embryonic shields. He says "The entire embryonic region (so far as visible) of the early blastoderm may be destroyed by the cautery and an apparently normal embryonic shield may arise by a process of regeneration." In describing these experiments Sumner also states that at this stage he had great difficulty in distinguishing just where the embryonic region of the germring was situated, so he was evidently operating upon an earlier stage than that dealt with in series 1 of my experiments. I have already pointed out that various parts of the blastoderm may be removed without interference with the development of normal but smaller embryos.

[3] Arch. f. Entwickl. Mech., Bd. 17, pp. 42-149, 1904.

Sumner's operations were probably on stages where the transition from a totipotence to an axial localization was in progress and it is not surprising that 10 out of 96 eggs operated upon should not have differentiated beyond the totipotent stage of the blastodisc, consequently normal embryos are to be expected.

If the posterior end of the embryonic shield is taken out, as shown in figure 2, the head end of the embryo will develop in a normal manner while the posterior portion of the embryo including the body and tail may be quite rudimentary or entirely wanting. The continued growth of the germring over the yolk and the closure of the blastopore takes place but the germring is apparently unable to contribute, after the operation, to the formation of a body or tail. The head end which develops is fairly normal in size and shape, its size depending upon the amount of tissue in the anterio-posterior axis of the shield that was not removed during the operation. Sumner likewise found that the destruction of the region near the posterior end of the embryonic shield prevented the development of the posterior part of the embryo.

In another series of experiments the anterior end of of the early embryonic shields was removed (fig. 3), with the resulting failure of development of the head end of the embryo while the body and tail developed normally up to about the time of hatching. The size of the body and tail likewise varies with the amount of tissue of the axis not removed during the operation.

In still another series of experiments a small area from the middle of the axis of the embryonic shield was removed, as shown in figure 4, with the resulting absence or rudimentary development of the posterior part of the head and cervical regions. The extent of the non-development in this region seems to vary directly with the amount and position of the tissue removed from the axis. The head and tail ends of the embryo seem to go on developing in a normal manner or nearly so though they may be separated from each other by a considerable distance. Such embryos may live for many days until after the time for the normal ones to hatch, though they themselves do not hatch.

If material is removed from the lateral region of such early embryonic shields, that is, to one side of the axial keel (fig. 5), there

is neither interference with normal development nor absence of any part of the embryo.

In still another series of experiments at this early stage of the embryonic shield, the germring was removed just lateral to the shield (fig. 6). In some of the experiments as much as one-fifth or one-fourth of the ring was removed on one side or the other. In a few minutes the gap closes and the yolk becomes more or less constricted along the region of the germring as the two ends of the wound come together, the amount of constriction varying with the amount of germring removed. The smaller germring evidently exerts considerable tension on the yolk. The loss of substance from the germring does not interfere with the formation of perfectly normal embryos as regards size and form. Morgan cut the germring and likewise got perfectly normal embryos. The removal of such large sections of the germring with the subsequent formation of a normal embryo would seem to indicate that the germring neither contained any especial organ forming substances nor is essential to the formation of the embryo.

Not only may a section of the germring be removed from one side of the embryonic shield but segments of the germring from both sides of the same shield may be removed without interference with the development of a normal embryo (fig. 7).

These various experiments show that concrescence plays very little or no part in the formation of the teleost embryo. The material which forms or directs the formation of the embryo is early concentrated along the axis of the embryonic shield, while the germring merely gives rise to the cells which form the covering to the yolk. This early concentration of the material for the embryo may perhaps be some sort of an abbreviated form of concrescence.

Sumner's[4] experiments 12, 22, 29, so far as indicating that the germring passes continuously into the embryo, would seem to me to indicate just the opposite, namely, that the germring probably does not contribute to the formation of the embryo body. The fact that the needle and the axis of the body approach each other is just exactly what one would expect to find taking place as the germring

[4] Op. cit.

decreases in circumference and the blastopore becomes smaller and smaller. The material in the germring is utilized in covering the yolk but otherwise there is no proof whatever of its contributing to the embryo. We are as fully justified, and perhaps more so, in claiming that the embryonic shield gives off cells to the germring as that the germring supplies material to the embryonic shield. The growing point of the embryo is at or near the posterior end of the axis of the embryonic shield and the material for the extension of the embryo is elaborated here in the primitive streak.

Operations on older stages of the embryonic shield

Operations on the older stages of the shield give results similar to those on the earlier stages. Removal of tissue from the anterior end of the shield produces various malformations of the head and often complete absence of that region of the embryo, (fig. 8, 8a, 9, 9a). The removal of small amounts of tissue at the tip of the shield often results in cyclopean monsters with varying degrees of fusion of the two eye rudiments, or the partial absence of one eye or of both eyes.[5] Likewise removal of tissue from the caudal end of the shield results in varying degrees of non-development of the caudal end of the embryo (figs. 10, 10a, 11, 11a).

As the embryonic shield elongates the extirpations of about the same amount of material from the posterior end of the axis gives rise to smaller defects in that part of the resulting embryo. This is exactly what would be expected if at or near the posterior end of the axis is located the growing point or node. There is of course continued differentiation and expansion along the entire axis of the shield as shown by the actual size of the extirpated area as compared with the much larger defects in the resulting abnormal embryos.

Just how far and how minutely predetermination and localization exist in the embryonic shield can only be determined by a more elaborate series of experiments. In experiment 15 (figs. 15, 15a) for example, the extirpation of a small amount of tissue at one side and extending into but not across the axis, resulted in producing

[5] W. H. Lewis, The experimental production of cyclopia in the fish embryo (Fundulus heteroclitus). Anat. Rec., vol. 3, no. 4, 1909.

an embryo which hatched and was able to swim abnormally. The brain on the side of the extirpated tissue was defective and the otocyst on that side was completely absent. Scarcely any two of the abnormal embryos are alike, they all show varying degrees of defectiveness. This is exactly what one would expect if there exists along the axis a definite localization, since it is almost impossible to plunge the needle into exactly the same point each time or to press out exactly the same amount of tissue even if the needle were plunged into the same point in a series of experiments.

In the older stages likewise, the removal of small areas along the median line of the shield gives rise to various kinds of defective embryos. If only a little of the superficial tissue is removed the resulting defect in the embryo is not very deep (figs. 12, 12a, 13, 13a, 15, 15a). After deeper and more extensive extirpation, the anterior and caudal portions of the embryo may be completely separated (figs. 14, 14a) yet they continue to develop in a fairly normal manner. As in operations on the anterior or posterior ends of the embryonic shield, the size of the defect varies with the amount of material extirpated and inversely with the length of the embryo. One may remove two or more such areas across the axis with the resulting development of three or four quite separate or partially separated segments of the embryo (figs. 16, 16a, 13, 13a). There is apparently no regeneration at all of the axial region of the embryonic shield and each individual region possesses great power of independent self-differentiation and this quite independently of the circulation, for in those experiments where the head end is separated from the caudal end of the embryo there is usually no circulation in the latter.

Removal of portions of the germring during any stage of the development of the embryonic shield apparently has no effect on the growth and development of the embryo.

Although the entire rudiment of the heart and head may be removed as in such experiments as 8, 9 (figs.8, 9, 8a, 9a) the blood vessels in the posterior portion of the body and even in the tail, develop and fill with red blood corpuscles. The capillaries likewise often develop over the yolk sac. There is a striking difference in the pattern which such capillaries form on the yolk sac in the

embryos without circulation as compared with the normal pattern. Although in the former the capillaries contain red blood corpuscles the capillary net work is very irregular and the capillaries themselves differ greatly in size. In the normal the mesh work is elongated and the capillaries are of about uniform size (figs. 17, 17a) The pigment cells over the yolk do not present the characteristic normal pattern in the abnormal embryos without circulation but are scattered irregularly like those observed by Loeb.

Many experiments were made on still older embryos after closure of the germring during the early stages in the segmentation of the mesoderm. As in the preceding experiments on the embryonic shield there was no regeneration of the parts removed and one is able with ease to produce headless or tailless monsters or embryos with head or tail ends separated by wide gaps (figs. 16, 16a).

CONCLUSIONS

Previous experiments indicate that there is no very definite localization either in the germdisc or blastodisc until about the time of the formation of the germring and embryonic shield.

With the formation of the embryonic shield very definite localization takes place along the axis of the shield coincident with the formation of the medullary cord. Removal of any portion of the axis of the embryonic shield results in very definite defects in corresponding parts of the embryo.

The embryonic shield does not possess the power of regeneration of the parts removed from the axis. Whether before this period of development the germdisc or blastodisc possess the power of regeneration is doubtful as there are no indications of any definite localization.

These experiments also show that the germring takes no part in the formation of the embryonic shield but merely serves for the formation of the covering of the yolk. The embryo then is not formed by concresence in the ordinary sense.

This method of experimentation affords an opportunity for experimental work on the central nervous system by the elimination of various portions at various stages. The development of the vascular system can also be studied experimentally.

PLATE 1

EXPLANATION OF FIGURES

1, 2, 3, 4, 5 Diagrams of early embryonic shields, 24 to 30 hours after fertilization; cross lines show the areas extirpated.

6, 7 Diagrams of embryonic shields, 30 to 36 hours after fertilization, to show extirpated portion of the germring.

8, 9 Diagrams of later stages of embryonic shields, 40 to 44 hours after fertilization; cross lines indicate amount extirpated.

8a Resulting abnormal embryo from experiment 8, 16 days after fertilization.

9a, 9b Abnormal embryos from experiment 9, 17 days after fertilization.

10, 10a Extirpation of posterior end of embryonic shield 36 to 40 hours after fertilization, and resulting abnormal embryo, 12 days after fertilization.

11, 11a Somewhat similar experiment to 10, 10a.

12, 12a Removal of small superficial area from the axis of an embryonic shield 46 hours after fertilization with resulting embryo, 15 days after fertilization.

13, 13a Removal of two superficial areas from the axis of an embryonic shield 46 hours after fertilization with resulting embryo, 8 days after fertilization.

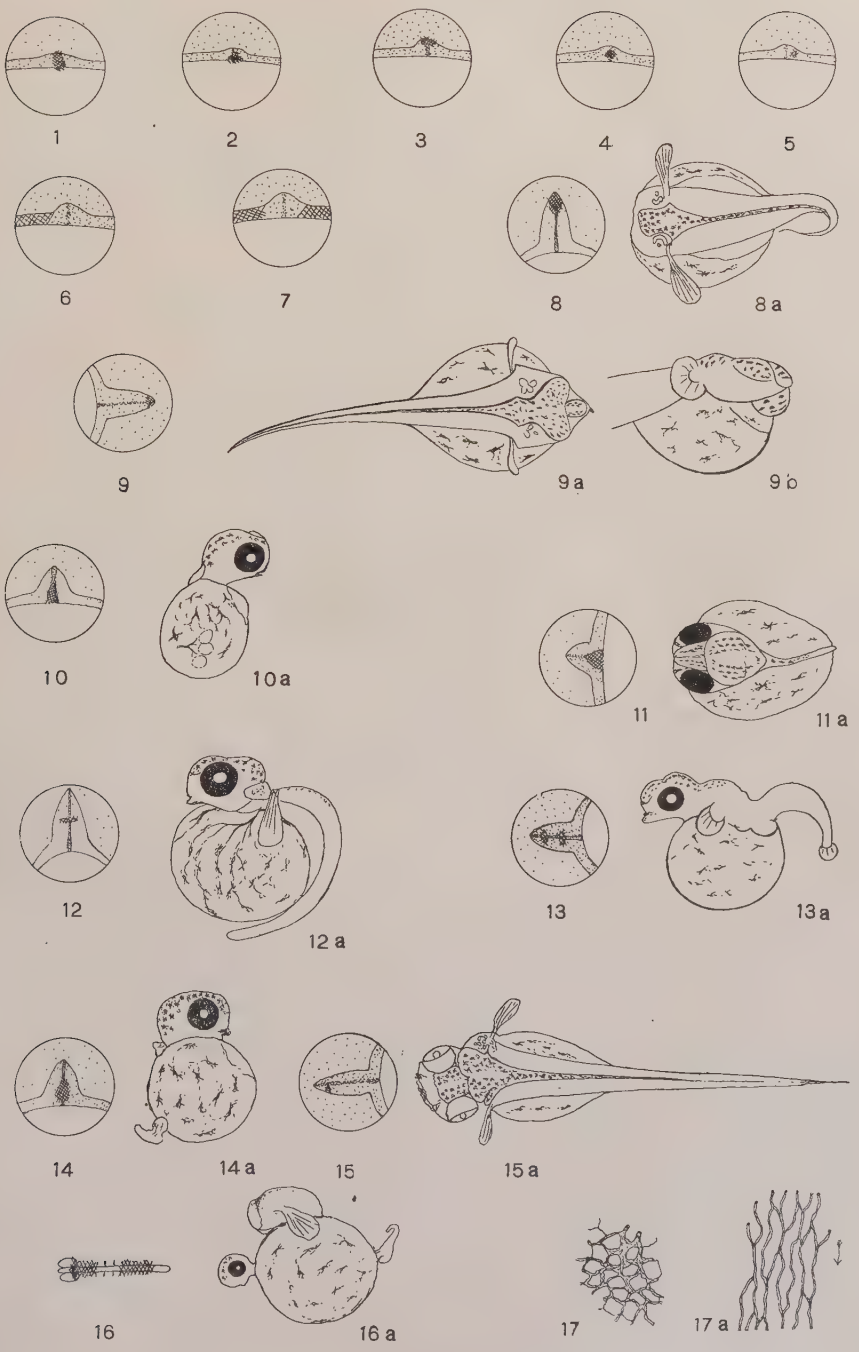
14, 14a Removal of a large deep area from the axis a shield 42 hours after fertilization with the resulting abnormal embryo 14 days after fertilization. The head and tail are completely separated by a wide area.

15, 15a Removal of a small lateral deep area from the axis of an embryonic shield 48 hours after fertilization with resulting defect of brain and absence of otocyst, 24 days after fertilization.

16, 16a Removal of two areas from the axis of an older embryo 70 hours after fertilization and resulting defects, 15 days after the fertilization.

17 Capillary plexus from yolk sac of embryo without circulation.

17a Normal capillary plexus from yolk sac.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ON THE ORIGIN OF THE ABDOMINAL LYMPHATICS IN MAMMALS FROM THE VENA CAVA AND THE RENAL VEINS

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It is a pleasure to have for study Mr. Kampmeier's able and valuable paper on the thoracic duct, as seen in one of my specimens, in the June number of The Anatomical Record. His direct dorsal view of a wax reconstruction of the jugular lymphatics brings out one point better than my profile reconstruction shown to the American Association of Anatomists in 1910. This point is the crossing of the thoracic duct to the right side which I have often, though not always noted, in injections of later stages. Moreover, his paper is a clear and excellent presentation of the theory of the growth of lymphatics by the addition of tissue spaces.

My own work on the thoracic duct is to appear in a forthcoming number of the Ergebnisse für Anatomie und Entwicklungsgeschichte, but I should like to present this preliminary note.

Lymphatics in mammals arise in two places, (1) from the anterior cardinal veins in the neck, (2) from the inferior vena cava and the adjacent veins of the Wolffian body or kidney. The budding of the jugular lymphatics is from the anterior cardinal veins near the duct of Cuvier but is not strictly limited to the anterior cardinal veins. In different forms the budding may extend along some of the adjacent veins, namely the posterior cardinal, the primitive ulnar and the capillaries that eventually make the root of the external jugular vein. The lymphatics which bud off from the inferior vena cava, which is a part of the large vein which connects the two Wolffian bodies, and from some of the veins in the edge of the Wolffian body grow in three directions. Those that grow ventral to the aorta make the retroperitoneal sac; those that grow caudalward, lateral to the aorta, make the iliac sacs; and those that grow dorsal to the aorta make the cisterna chyli and the lower part of the thoracic duct. There are therefore two pairs of symmetrical primary sacs in mammals, the jugular and the iliac,

and two median sacs, the retroperitoneal and the cisterna chyli. It might be said that there are three different sacs in mammals, the jugular (paired), the iliac (paired) and the retroperitoneal (unpaired) and these three sacs are connected with each other by the cisterna chyli and the thoracic duct. However as I shall define primary lymph sacs as those which bud directly off from the veins, it is necessary to include the cisterna chyli with the primary lymph sacs. The primary lymphatic sacs and the thoracic duct make the primary lymphatic system.

The thoracic duct arises in part as a down growth from the left jugular lymph sac and in part from a plexus of lymphatics which buds off from the veins of the Wolffian body. While the thoracic duct is incomplete, it is excessively hard to inject. The specimen which I loaned to Mr. Kampmeier is not a perfect injection since it has extravasations; it is, however, the first and until the past month the only injection ever made of a mammalian thoracic duct before it is complete. It was made indirectly through the jugular sac. I filled the sac with ink and then bent the head forward and to my surprise the ink shot into the thoracic duct. As will be readily seen an injection into the jugular sac almost invariably runs by the path of least resistance or the physiological path into the veins and does not back into the thoracic duct. Perfect injections of lymphatic capillaries without any extravasations can be made, as for example Mrs. Clark's injection of the jugular lymphatic plexus in the chick shown on page 263 of the June number of The Anatomical Record for this year, 1912, but to obtain perfection it is necessary to watch the entire field of injection under the microscope in order to regulate the pressure.

Mr. Kampmeier's figure 1 shows the jugular portion of the thoracic duct which arises as sprouts from the jugular sac. These sprouts form a plexus dorsal to the esophagus from which ducts grow downward. The shorter left duct follows the vein to the root of the heart and I think subsequently grows to the left side of the heart and the left lung. A longer duct crosses to the right side and is the upper thoracic part of the thoracic duct.

The abdominal portion of the thoracic duct in the pig buds off from the two sides of the large vein which connects the Wolffian bodies and forms part of the vena cava and from the large veins in the capsule of the Wolffian body itself. Dr. and Mrs. E. R. Clark have shown in the June number of The Anatomical Record for 1912 that the lymphatics for the posterior lymph hearts of the chick bud off from the coccygeal vein and its branches, and these lymphatic buds are filled with stagnant

blood by a back flow from the parent vein. These lymphatic buds first make a plexus out of which the lymph heart is formed. In the chick the lymphatics while they are still in the blood filled stage grow out to form the peripheral capillaries in the skin. Moreover they are closed vessels, first because the blood in them is held within the contour of the vessel, and secondly because a little ink injected into one vessel will fill the entire lymphatic system and empty into the veins without any extravasation.

In reworking all of the sacs in the pig, I have found that the primary buds are packed with blood (fig. 7 in 14) and that the blood empties gradually so that the sacs are subsequently partially filled and finally empty. The jugular lymphatics bud off from the jugular veins making the well known jugular sacs. All the rest of the lymphatics in the pig bud off from the mesonephritic veins. The retroperitoneal sac comes off from the ventral surface of the mesonephritic vein in the midline of the embryo. I found out the fact that the abdominal part of the thoracic duct comes from the same vein by direct puncture of the cisterna chyli in the blood filled stage. I saw the ink enter the mesonephritic vein. I have three injections of the abdominal sacs or rather plexuses made in embryos 23 mm. long. No one of them is perfect and none shows the cisterna chyli since the ink around the point of injection obscures it. They all show that the beginning thoracic duct, two symmetrical plexuses along the edge of the Wolffian body and the retroperitoneal sac connect. I shall subsequently describe these injections more in detail when I can also present views of the veins of the region as well. For the present I want to describe the blood filled lymphatic buds as they appear in 23a which Kampmeier studied, as well as in another specimen from the same litter and in a human embryo of about the same stage.

In the series 23a, which measured 23 mm. in its greatest length in the fresh specimen the blood packed lymphatic buds are to be seen in sections at the level of the median mesonephritic vein. They are in the angle between the vein and the medio-dorsal edge of the Wolffian body. These buds while still packed with blood form (1) the iliac lymphatics and (2) the cisterna chyli. In a series measuring 22 mm., I have found the earliest blood filled iliac buds arising from the large veins in the capsule of the Wolffian body, as well as the dorso-lateral surface of the mesonephritic vein near the Wolffian body. In 23a the iliac plexus of blood packed lymphatics has grown caudalward along the dorso-medial border of the Wolffian body as far as the hilum of the

permanent kidney. This plexus of blood filled lymphatic capillaries is spreading out in the capsule of the kidney and in later stages I have injected a plexus of lymphatic vessels from the iliac sac into the hilum of the permanent kidney around its pelvis. This explains why the permanent kidney is supplied by iliac lymphatics, while on the other hand the retroperitoneal sac spreads into the ventral capsule of the Wolffian bodies as far as the edge of the reproductive glands. Their lymphatics therefore grow from the retroperitoneal or prae-aortic sac.

The iliac sac is not complete in 23a; the iliac plexus grows down to a point exactly opposite the bifurcation of the aorta where it swells into a sac which I first identified as the posterior sac in 1901 in an embryo pig 25 mm. long. From this enlarged end grow three main groups of lymphatics, (1) an abundant plexus of vessels surrounding and following the umbilical arteries, (2) the femoral and (3) the ilio-lumbar vessels which together drain the leg and abdominal wall.

The cisterna chyli is not present in 23a, but in an embryo 23b from the same litter as 23a, the blood filled plexus which arises on either side in common with the iliac plexus has arched across the midline, dorsal to the sympathetic ganglia and the aorta. Immediately dorsal to the aorta at the level of the anlage of the adrenal bodies the blood filled lymphatics have enlarged into a cisterna chyli. In my article in the Ergebnisse I have shown this important point, that all of the primary lymphatics bud into non-vascular zones; if the non-vascular areas are large, the sacs are large, as for example the jugular and the retroperitoneal sacs in the pig; if the non-vascular areas are small, the sacs are small, as for example the iliac sac and the cisterna chyli in the pig. The cisterna chyli develops opposite the adrenal anlage just at the tip of the developing azygos veins. Caudal to this point the segmental venous plexus drains through the Wolffian body and the transition zone is a small non-vascular area. Tracing the thoracic duct by the presence of the blood, the vessels have not yet grown beyond the region of the adrenal bodies, the blood filled plexus has, however, grown caudalward dorsal to the aorta to a point opposite the hilum of the permanent kidney.

The rest of the thoracic duct forms rapidly, and is probably complete at the stage measuring 25 mm. I have completely reconstructed it at 27 mm. The lower segment is always characterized by being an exceedingly abundant plexus which surrounds the aorta as is shown in Pensa's figures (11), while the portion from the heart to the neck is often a single trunk or at least a few vessels. In a third specimen meas-

uring 23 mm. in which I washed out the blood vessels with Locke's solution, the abdominal lymphatics are partially filled with blood and the veins are empty. This shows that the blood packed stage is a short one. In this specimen lymphatics from the retroperitoneal sac are growing around the aorta to connect with the cisterna chyli. Subsequently the main connection is around the cerebral surface of the adrenal. All of the abdominal sacs become connected with each other.

A new embryo in the Mall collection, No. 460, measuring 21 mm. is of great interest in connection with the abdominal lymphatics. A careful reconstruction of this specimen with abundant illustrations will be of value, but for the present I can only give a brief description. I injected the embryo with India ink through the umbilical artery while the heart was still beating. The vascular injection is almost perfect. The embryo was then put directly into bichloride-acetic and the fixation is excellent.

In marked contrast to the embryo pig of about the same length the Wolffian bodies are disappearing, being pushed caudalward by the growing permanent kidneys. The cerebral pole of the Wolffian bodies, now far to the side, lies opposite the large median vein which still drains the Wolffian bodies. This vein is now markedly assymmetrical, owing to the enlargement of the right side of it, due to the vena cava. From the sides of the median vein extends a plexus of veins to the permanent kidneys.

From the ventral surface of this vein in the midline is a group of blood filled lymphatic buds, lying in the root of the mesentery. This area is very small in the human embryo, in marked contrast with the large area of the pig, and the human retroperitoneal or prae-aortic sac is consequently of small size. It is of considerable interest to note that the connective tissue near these lymphatic buds has wider spaces than along the rest of the border of the vein. They can, however, be distinguished from the lymphatics. Some lymphatics are growing from the dorsal surface of the median vein, and, pushing between the masses of cells of the sympathetic ganglia, have reached the ventral surface of the aorta.

The iliac lymphatic sacs are of great interest. They are farther advanced than the retroperitoneal sac, because (1) they are larger and (2) they are only partly filled with blood. They arise not from the veins of the Wolffian body but from the veins of the permanent kidney. Along the dorso-medial border of the kidneys between the kidney and the segmental veins are two long sacs, the one on the left being about 1.8

mm. long. The left sac reached the bifurcation of the aorta; the caudal part of the right sac is less developed, and the place for it is small, on account of the size of the vena cava. Instead of a sac there are some blood packed buds along the vena cava itself.

The cerebral ends of the iliac sacs meet in a small plexus of blood filled vessels dorsal to the aorta opposite the adrenal bodies. This marks the position of the future cisterna chyli. The abdominal part of the thoracic duct can be traced for some distance in the sections. The trunk for the jugular sac is present but I do not think that the two parts have met. Another point of great interest in the specimen is that it shows many areas of widened tissue spaces along the aorta. They can, however, be distinguished from the lymphatics.

The question at issue between Mr. Kampmeier and myself is the method of growth of the thoracic duct between the two anlagen, the jugular and the renal segments. I admit at once that I have not seen the duct grow in the living form, nor have I mastered the difficulties of injecting it so as to demonstrate its gradual growth from two venous anlagen. The isolated spaces shown in Mr. Kampmeier's figure 1, I submitted to most careful study before the meeting in 1910 and discussed them there; they are endothelial lined and I believe them to be lymphatics. I believe that adequate injections would show that they connect with the rest of the system. It was these vessels which made me consider whether the origin of the thoracic duct was not from a series of vessels from the azygos veins, but this point has now been cleared up by finding that the cisterna chyli arises as blood filled buds from the mesonephritic or renal veins. The fact that the injection mass extravasated just above the long apparently isolated lymphatic on the right side is not an unsurmountable difficulty, for it has been noted by a long list of observers among whom are Sigmund Mayer (9), Ranvier (12), MacCallum (8), Bartels (1) and myself (13) that it is an especial characteristic of lymphatic capillaries that tiny sprouts in which extravasations may readily occur often lead to wide sacs or vessels. In fact both Stricker (16) and Sigmund Mayer have seen blood capillaries contract down to a thread in a living tadpole's tail, so it is known that endothelium is contractile.

In regard to the method of growth of the intermediate division of the thoracic duct, I know that the peripheral lymphatic capillaries grow by budding of their endothelial wall, having seen them in the living tadpole, I know that the lymphatics in the chick bud off from the veins and grow to the peripheral capillaries in skin as closed endothelial vessels

having seen them in the Clark's specimens. I know as nearly as possible without actually seeing the process in the living form, that the thoracic duct begins in mammals as a down growth from the jugular sac and as an up growth from the cisterna chyli which comes from the mesonephritic or renal veins, I therefore do not believe that its further growth is by a process different from the rest of the lymphatic and blood vascular systems.

The discovery that all the abdominal lymphatics in mammals come from the veins of the Wolffian body or kidney brings out the significance and the importance of Silvester's (15) beautiful injections in South American monkeys. He showed that in this one form the renal connections are permanent. It also allows us to consider the question of the relation of the mammalian lymph sacs to the lymph hearts and sacs of the amphibia. The anterior lymph hearts of the amphibia bud off from the vertebral veins (Knower (7) and Hoyer (5)) and as Knower has shown gets its muscle from the myotomes; the posterior lymph hearts of amphibia and birds arise from the coccygeal veins, they lie against the myotomes and have muscle in their walls. The anterior lymphatics of birds and mammals arise from the anterior cardinal vein and do not develop muscle. The abdominal lymphatics of mammals bud off from the renal veins and form three sacs, the retroperitoneal, the iliac and the cisterna chyli. These sacs are like the amphibian lymph hearts in their most fundamental point, namely that they bud directly from the veins, they differ from them in position not lying against the myotomes and in not developing muscle. They are not analogous to the amphibian lymph sacs which are secondary structures, transformed from lymph ducts. The mammalian sacs therefore might be called primary lymphatic sacs. As a matter of fact the following is the embryological classification. Primary lymph sacs: (a) those which develop muscle in their wall, forming true lymph hearts, for example the anterior and posterior lymph hearts of amphibia and the posterior lymph hearts of birds; (b) those which do not develop muscle, but become partially transformed into lymph glands, for example the jugular lymph sacs of birds and mammals and the mesonephritic (renal) lymph sacs of mammals. In a word, primary lymph sacs may become lymph hearts or lymph glands.

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ON THE DEVELOPMENT OF THE PREVERTEBRAL (THORACIC) DUCT IN TURTLES AS INDICATED BY A STUDY OF INJECTED AND UNINJECTED EMBRYOS

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EIGHT FIGURES

The problem of the development of the lymphatics has been the cause of much discussion and of a great deal of investigation during recent years. Most of the investigations have, however, been limited to the study of this problem in mammals where the close relation between the developing lymphatics and certain embryonic veins is such as to cause considerable confusion. From the conflicting theories which have arisen, partly as the result of the methods of investigation and partly from the complexity of the mammalian type of lymphatic development, it is evident that the study of a simpler type is desirable. It seemed to the writer while tracing the development of certain veins in the reptiles that the loggerhead turtle presents peculiar advantages for the study of the lymphatics. The adult lymphatic system in turtles is of a comparatively simple and generalized type and the embryonic anlagen are quite easily distinguished from the blood vessels. While the anatomy of the adult lymphatic system of turtles has been quite carefully studied by Bojanus ('19), Panizza ('33), Müller ('33) and others, its development until recently[1] appears to have been entirely neglected.

[1] Literature on the development of the lymphatics in reptiles: George S. Huntington; The development of the lymphatic system in reptiles. Anat. Rec. vol. 5, 1911. Frank A. Stromsten: The lymphatic system of turtles. Science, vol. 31, 1910 a. The development of the posterior lymph hearts of the loggerhead

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In order to facilitate comparison with the results obtained from my study of the development of certain lymphatics in the loggerhead turtle, a very brief statement of the two leading theories of lymphatic ontogeny will be given at this point.

(1) *The theory of the venous origin of the lymphatics (Ranvier and Sabin):*

The lymphatics grow from the various primary lymph sacs by the sprouting of endothelium and gradually spread over the body. The primary lymph sacs arise from the veins and their endothelial lining is derived from the venous endothelium. . . . Lymphatics are modified veins. They are vessels lined by an endothelium which is derived from the veins (Sabin).[2]

(2) *The extra-intimal theory of Huntington and McClure:*

The peripheral general lymphatic channels appear to be developed by the confluence of spaces independent of the venous system, although closely associated with the same. . . . They begin as minute extra-venous vacuoles, closely applied to the surface of the veins which they accompany. They enlarge as the lumen of the vein diminishes. They become confluent with each other, but never contain red blood cells, nor do they communicate with the blood channels (Huntington).[3]

It should be borne in mind, however, that lymph channels may be established in the body independently of the veins and that the extra-intimal replacement of a decadent vein by a lymph channel is a secondary procedure, in the sense, that the pathway of the decadent vein merely offers a course of least resistance to the centripetal flow of lymph from the periphery to the point or points where it enters the venous system (McClure).[4]

turtle. Abstract in Proc. Iowa Academy of Science, vol. 17 1910 b; A contribution to the anatomy and development of the posterior lymph hearts of turtles. Publication No. 132 of the Carnegie Institution of Washington 1910 c; On the relations between the mesenchymal spaces and the development of the posterior lymph hearts of turtles. Anat. Rec. vol. 5, 1911 a; The development of lymph channels in turtles by the fusion of mesenchymal spaces. Science. N. S., vol. 34. Proc. Iowa Academy of Science, vol. 18, 1911 b.

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[4] Charles F. W. McClure: The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat (Felis domestica). Verhandlungen der Anatomischen Gesellschaft, August, 1910 p. 106.

These diametrically opposed theories seem to be the result, in part at least, of the methods employed in the investigations. Those who contend for the venous origin of the lymphatics have been influenced mainly by the injection method while the other theory has been based principally on a careful study of serial sections.

It is the purpose of this paper to establish the fact, chiefly by the study of sections of injected turtle embryos, of the presence of independent lymph spaces which lie beyond the field of the injected vessels, and that these lymph spaces by fusion aid in the formation of a peri-aortic lymphatic plexus which later becomes directly transformed into the prevertebral (thoracic) duct. A very brief synopsis of this paper was presented before the Iowa Academy of Science, April, 1911 (Stromsten).[5]

The work for the most part has been carried on in the Laboratories of Animal Biology at the State University of Iowa. All of the embryos of the loggerhead turtle were collected by the writer at the Marine Laboratories of the Carnegie Institution of Washington at the Dry Tortugas, Florida. Embryos of land turtles, snapping turtle, box-turtle, and painted turtle were also obtained from various localities in the United States. The results here given are based entirely on the conditions found in the sea-turtle, although frequent comparisons were made with the various land turtles during the progress of the work.

On account of the great curvature of the body at certain stages, the usual measurements do not give accurate data as to the age of the embryo. Since the exact time of laying is known in all of the eggs studied in this investigation, the age in days is given in every case. The most important stages for the present study are from twenty-four to thirty-one days. Table 1 will give the average crown-rump measurement of a number of individuals for each stage.

Numerous fixing agents were tried, but the most satisfactory results were obtained by using a mixture of chromic acid, glacial

[5] The development of lymph channels in turtles by the fusion of mesenchymal spaces. Science, N. S., vol. 34, Proc. Iowa Academy of Science, vol. 18, 1911 b.

TABLE 1

DAYS OLD	MEASUREMENT	DAYS OLD	MEASUREMENT
	mm.		*mm.*
$24\frac{1}{3}$	15	27	18
25	16	$27\frac{1}{2}$	$18\frac{1}{2}$
26	17	$28\frac{1}{3}$	19
$26\frac{1}{2}$	$17\frac{1}{2}$	$29\frac{1}{4}$	$19\frac{1}{2}$
$26\frac{3}{4}$	18	30	20

acetic acid and 40 per cent formaldehyde.[6] Bouin's picro-formol, Carnoy's fluid and several other fixing fluids gave very good results, but corrosive sublimate mixtures, as a rule, were not satisfactory for sea-turtle embryos. In the matter of killing and fixing the greatest care was always exercised. In the later stages it was deemed advisable to narcotize before fixation in order that the delicate mesenchymal tissue might not be injured by the twisting and squirming of the embryo when first placed in the fixing solution.

In every process, from the fixation of the embryo to the final mounting of the sections under the cover glass, the greatest care was exercised to prevent any shrinking or distortion of the tissues. Sections were cut from 10μ to 50μ in thickness, depending on the size of the embryo. Transverse, frontal, and sagittal sections were made of all important stages. Embryos older than thirty-eight days were imbedded in celloidin and mounted on lantern slide covers.

A very complete series of injected embryos was obtained. The blood vessels were usually injected through the vitelline vessels but, in some cases, directly through the heart. The lymphatic injections were made with India ink, according to the method of Knower, in the cervical and post-iliac regions. When successful, the jugular lymph-sac or the posterior lymph-heart, as the case might be, with their attached lymphatics were well filled with the injecting fluid.

[6] Chromic acid, 1 per cent aqueous solution.....64
Glacial acetic acid.....4
Formaldehyde, pure (40 per cent).....32

The sections were stained with Delafield's or Gage's haematoxylin, iron haematoxylin, or in toto with alum or borax carmine. The counterstains most frequently used were orange-G (slightly acidulated), van Gieson's acid fuchsin, eosine-aurantia-orange-G, and Mallory's connective tissue stain. Wax and profile reconstructions were made of important regions of the embryos most studied. Camera lucida drawings were made of all stages and the more important of these were checked with photomicrographs.

The most satisfactory as well as the most convenient way of approaching the problem of the development of the lymphatic system is to commence with the study of a stage of development in which the parts under consideration are so completely formed as to leave no doubt whatever as to their identity. From this stage the history of development may be traced backward through the earlier stages and forward to the adult condition without any serious difficulty. This is the method that has been pursued in the preparation of this paper.

In an embryo of the loggerhead turtle of thirty-eight days incubation there is no difficulty whatever in distinguishing the prevertebral lymphatic duct. It more or less completely envelopes the dorsal aorta throughout its entire length. It arises at the caudal extremity of the aorta from a rather compact plexus of lymph channels which invests the great branches of the aorta in the pelvic regions. At this stage it is paired throughout the greater part of its length. The paired anlagen, which run along the dorsolateral aspects of the single aorta, are frequently connected by dorsal and ventral anastomoses. Further forward, near the bifurcation of the aorta, the paired anlagen unite to form a single sac which extends a short distance along the dorsal surface of the aorta. This sac then bifurcates with the aorta, following the aortic arches to open finally into the jugular lymph-sacs. The entire duct is somewhat Y-shaped with the two arms bent ventrally hookwise. It receives tributaries from the anterior and posterior limbs and from the region of the thyreoid and post-branchial bodies, also from the periarterial lymphatics of the main branches of the aorta. The right and left arms of the duct almost completely encircle their respec-

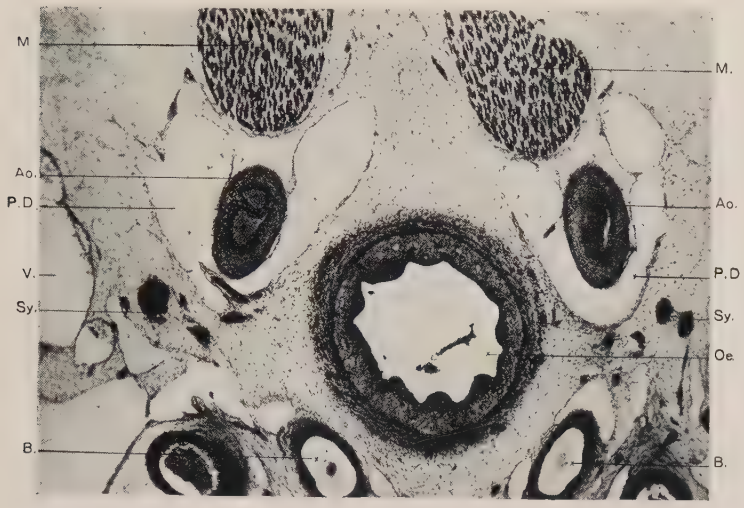
tive aortic arches so as to form tube-like investments through which these arteries run. The walls of these lymphatic tubes are not complete in the sense of entirely shutting off the aortae from the surrounding tissue. A wax reconstruction of this stage shows the tubes to be really a lymphatic plexus in which the meshes are very small and few in number, while the channels are broad and flattened. Reconstructions of the thirty-four-day stage show that this condition obtains in the region adjacent to the lymph-sacs only. Further caudad the network arrangement of the lymphatics becomes more apparent. In the region of the aortic bifurcation and beyond it, this network is quite open but continues unbroken to the caudal region. At the thirty-two-day stage, the peri-aortic plexus (fig. 1 *P.D.*) is more open dorsally. Ventrally and laterally, however, the plexus is still quite compact. At thirty-one days' incubation the peri-aortic lymphatic plexus is an open network of lymph channels throughout its entire extent.

Careful studies made of a large number of specimens of these and of intermediate stages reveal every step in the transformation of this peri-aortic lymphatic plexus into the prevertebral duct of later stages. The evidence for this change is positive and direct and is in perfect accord with the results of Polinski, Mierzejewski and others. Our study thus far will enable us to make our first positive statement with respect to the development of the prevertebral lymphatic duct in turtles.

The prevertebral lymphatic duct of turtles is formed by the enlargement and fusion of the individual elements of a peri-aortic network of lymph vessels.

Fig. 1 Loggerhead turtle, thirty-two days, series 228, × 60. Photomicrograph of a section taken through the region of the dorsal aortae to show an advanced stage in the development of the prevertebral lymphatic duct. *M.*, muscle; *Ao.*, aorta; *P.D.*, peri-aortic lymphatic duct; *Sy.*, sympathetic nervous system; *Oe.*, oesophagus; *B.*, bronchus; V., vein.

Fig. 2 Drawing of a wax reconstruction of the lymphatic plexus of an uninjected loggerhead turtle embryo of twenty-nine days, the continuous portion of the plexus is shown in black. This is followed by a series of isolated spaces.


M.
M.
Ao.
P.D.
Ao.
V.
P.D
Sy.
Sy.
Oe.
B.
B.

1

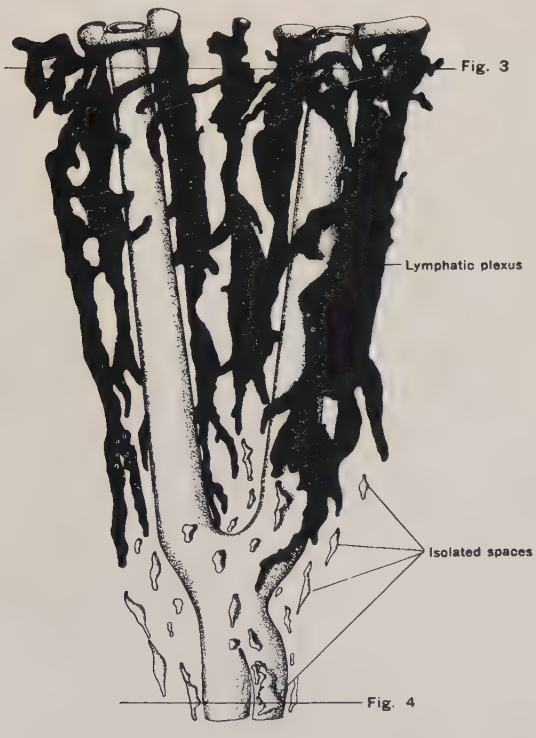
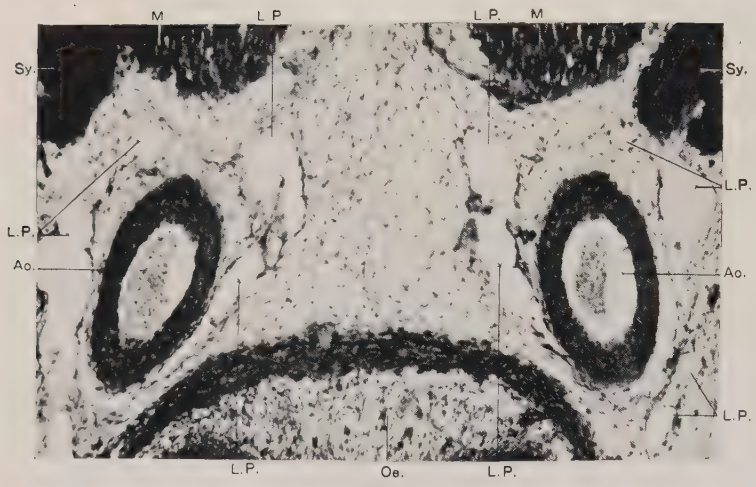


Fig. 3
Lymphatic plexus
Isolated spaces
Fig. 4

2



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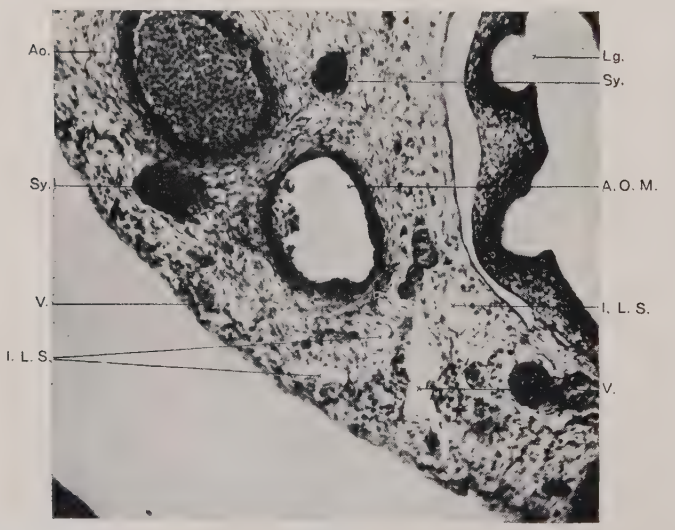


4

Fig. 3 Loggerhead turtle, twenty-nine days, series 640u., × about 170. Photomicrograph of a section taken through the anterior region of the plexus reconstructed in figure 2. *Ao.*, aorta; *L.P.*, peri-aortic lymphatic plexus; *M.*, muscle; *Sy.*, sympathetic nervous system; *Oe.*, oesophagus.

Fig. 4 Loggerhead turtle, twenty-nine days, series 640u., × about 170. Photomicrograph of a section taken through the region designated in figure 2. *Ao.*, aorta; *Sy.*, sympathetic nervous system; *V.*, vein; *I.L.S.*, isolated lymph spaces; *A.O.N.*., omphalo-mesenteric artery; *Lg.*, lung.

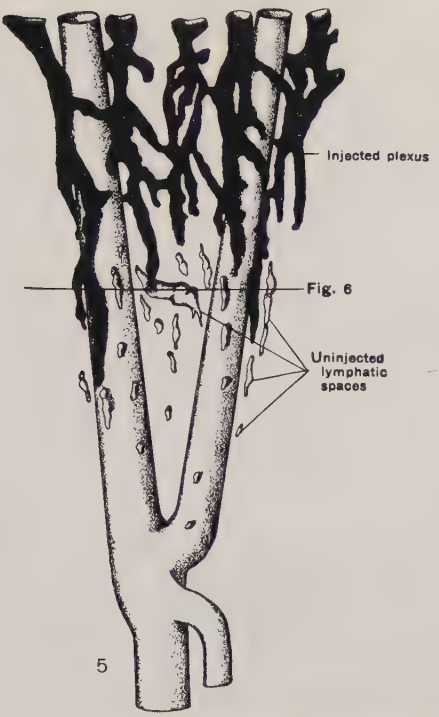


Fig. 5 Drawing of a reconstruction of the peri-aortic lymphatics of an injected loggerhead turtle embryo of $28\frac{3}{4}$ days incubation. Injected portion in black, followed by a series of uninjected, isolated anlagen.

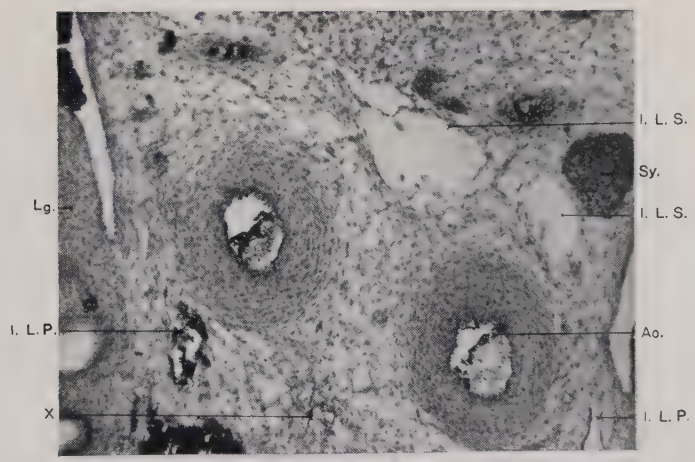
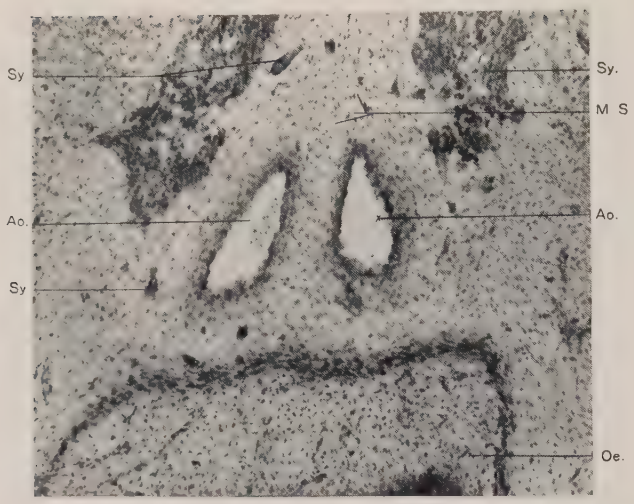


Fig. 6 Loggerhead turtle, $28\frac{3}{4}$ days, series 640i., × about 170. Photomicrograph of a section taken through the region designated in figure 5, *Ao.*, aorta: *I.L.S.*, isolated lymphatic space; *Sy.*, sympathetic nervous system; *I.L.P.*, injected lymphatic plexus; *X.*, extravasation of India ink from an injected lymphatic; *Lg.*, lung.



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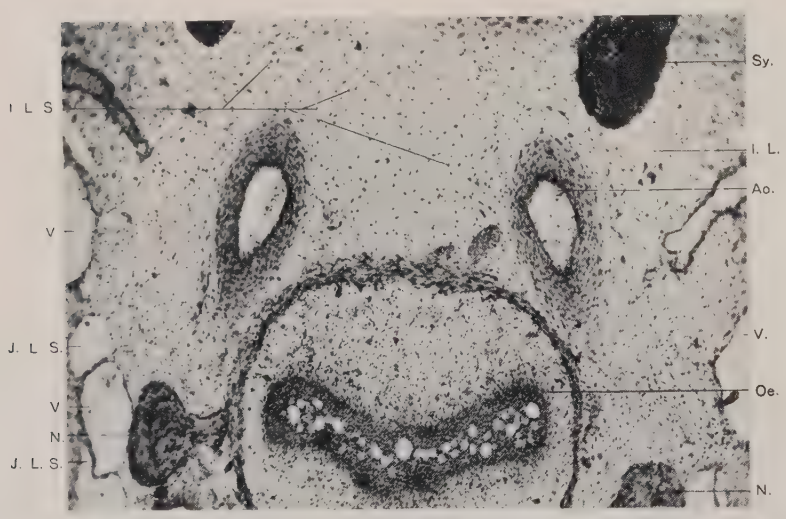

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Fig. 7 Loggerhead turtle, twenty days, series 230, × about 170. Photomicrograph of a section taken through the region of the aortae to show the compact nature of the peri-aortic mesenchyme. *Ao.*, aorta; *Sy.*, sympathetic nervous system; *Oe.*, oesophagus; *M.S.*, intercellular mesenchymal spaces or vacuoles.

Fig. 8 Loggerhead turtle, twenty-four days, series 164, × about 60. Photomicrograph of a section taken through the region of the aortae to show the loosening up of the peri-aortic mesenchymal tissue during the first stages of lymphatic development. *Ao.*, aorta; *I.L.S.*, independent lymphatic spaces at an early stage of development; *Sy.*, sympathetic nervous system; *V.*, vein; *Oe.*, oesophagus; *J.L.S.*, jugular lymph-sac; *N.*, vagus nerve.

The origin of this lymphatic network, however, is the point at issue. Early in the year 1910 the writer became convinced from the study of sections of uninjected embryos that this peri-aortic plexus was formed by the enlargement and fusion of independent mesenchymal spaces. This view was expressed in a paper read before the American Society of Zoölogists, Central Section, at its spring meeting (Stromsten '10). In order, however, to test the results further, it was deemed advisable to collect a new series of embryos and inject the haemal and lymphatic systems by the delicate method perfected by Knower ('08) and others. The following results, for the most part, are based on a study of these injected embryos.

Figure 2 is a drawing of a wax reconstruction made of a portion of the aorta and the developing peri-aortic lymphatic plexus of an uninjected turtle embryo of twenty-nine days' incubation. The peri-aortic lymphatic plexus in this stage extends from the jugular lymph-sac region to a short distance beyond the bifurcation of the aorta. It is continuous and easily traced along its entire length. Figure 3, *L.P.* shows a cross section taken through the anterior region of the peri-aortic plexus. Following this continuous plexus there is a series of isolated spaces of somewhat irregular shape as shown in the reconstruction (fig. 2), and in cross section in figure 4, *I.L.S.* That these spaces are absolutely independent of the plexus was determined to the satisfaction of the writer from the study of uninjected embryos. However, to leave no doubt whatever in the matter a number of injected turtle embryos were sectioned and studied.

Figure 5 shows a reconstruction (ventral view) of an injected embryo of $28\frac{3}{4}$ days' incubation. This specimen had both arteries and veins injected with India ink by forcing the fluid into the beating heart. The jugular lymph-sacs were also injected after the manner described by Sabin for pigs, using Knower's ingenious device for forcing the India ink into the vessels. In the embryo under consideration the lymphatic plexus was injected as far caudad as it had been developed. Extravasations occurred at various points as is shown in photomicrograph, (fig. 6, *X*). Beyond the injected portions, the sections show the undoubted

presence of isolated lymphatic spaces of considerable size (fig. 5.) The larger of these spaces are in the region dorsal and dorso-lateral to the aortae. A section taken through the largest of the independent spaces found in this specimen is shown in figure 6, *I.L.S.* Smaller independent spaces are found scattered along the aortae almost as far caudad as the origin of the omphalo-mesenteric artery (fig. 5).

It is thus evident, that the two recognized methods of investigation which have been applied to the study of the genesis of the lymphatics, agree in demonstrating in turtle embryos, that beyond the farthest portion of a continuous lymph channel definite independent spaces are met with in the mesenchyme which are not connected with the continuous system of vessels. Since these spaces lie in the actual pathway of the developing lymphatics and no where else and, at the same time, immediately precede the appearance of a continuous system of lymph vessels in this region, there can be little doubt but that they enter directly into the formation of the peri-aortic lymphatic plexus.

In view of these facts it seems justifiable to make a second definite statment regarding the development of the prevertebral lymphatic duct in turtles.

The development of the peri-aortic lymphatic plexus in the loggerhead turtle is immediately preceded by the formation of isolated, independent spaces. These spaces are always found in the exact area that this plexus is to occupy and nowhere else. They cannot be injected, even though the lymph-sacs and the peri-aortic plexus are completely filled. There is every reason to believe that they fuse with and finally form part and parcel of the peri-aortic plexus.

There is still one more question that should be considered: Are these isolated spaces true mesenchymal spaces, or, as shown in section, do they represent the 'Mayer-Lewis anlages' of Sabin in the sense that they are sections of functional blood capillaries, or sections of detached portions of degenerating veins? As far as the turtle is concerned, there appears to be no possibility that these independent spaces may have been derived from the veins, since the territory in which these spaces develop is notably free of veins.

It is further possible to trace clearly and without a doubt the development of the isolated lymphatic spaces from the original intercellular spaces. Figure 7 shows a section taken through the region of the aortic arches of a loggerhead embryo of twenty days' incubation. The mesenchyme surrounding the aortae (*Ao.*) is quite dense and compact. The nuclei are closely crowded. Intercellular spaces are very minute. Blood capillaries are rarely met with, but when present are clearly defined and are usually filled with blood. The peri-aortic mesenchyme does not appear different from the mesenchyme elsewhere in the body.

At twenty-three days, however, we find marked changes. The mesenchyme in the peri-aortic region shows a decided loosening up of the cells. The rapid enlargement of the intercellular spaces has forced the cells far apart. The widely separated nuclei are surrounded by a narrow rim of cytoplasm which is drawn out into long, slender filaments connecting the several cells into a syncytium. The comparatively large intercellular spaces of this loose, spongy mesenchyme are frequently filled with wandering leucocytes to such an extent as to mask the real structure of the tissue. These mesenchymal spaces (fig. 8. *I.L.S.*) continue to increase rapidly during the twenty-fourth and twenty-fifth days. Certain of the slender cytoplasmic processes seem to break or degenerate so that it is not at all uncommon to find them projecting into the mesenchymal cavities. The cavities themselves do not have a definite endothelial wall but are lined by cells indistinguishable from those of the surrounding mesenchyme. These cavities, or lymphatic lacunae, as they may be called, are real morphological structures; they are not artifacts. They are invariably found in all turtle embryos of this stage of development and are always in the direct pathway of developing lymphatics. That they actually form an intrinsic step in the development of the lymphatic plexus, instead of merely furnishing the path of least resistance for its onward progress, is shown by the fact that it is possible to trace the transitional stages between these lacunae and the simple intercellular spaces (figs. 7 and 8) on the one hand, and the isolated lymphatic anlagen (fig. 6 *I.L.S.*) on the other. It is, in fact, impossible to tell just

when a lacuna ceases to be a lacuna and when it becomes an 'isolated space.'

The formation of the peri-aortic lymphatic plexus in the loggerhead turtle is always preceded by a vacuolation of the mesenchyme in the exact region to be occupied later by this plexus. The intercellular spaces thus formed enlarge and fuse together to form lymphatic lacunae. At a later stage the lacunae acquire an endothelial lining and become the isolated anlagen of the lymphatic plexus.

To sum up: The developing prevertebral lymphatic duct in turtles passes through the following stages:

1. Stage of vacuolation of the periarterial mesenchyme (twenty-three to twenty-six days), figures 7 and 8.

2. Stage of enlargement and confluence of mesenchymal vacuoles to form lymphatic lacunae (twenty-five to twenty-seven days), figure 8.

3. Stage of the enlargement of lacunae and the acquirement of endothelial walls to form isolated lymphatic anlagen (twenty-seven to thirty days).

4. Stage of the formation of a lymphatic plexus by the confluence of isolated anlagen (twenty-seven to thirty days), figures 2, 3, 4, 5, and 6.

5. Stage of the transformation of the lymphatic plexus into the prevertebral lymphatic duct (fig. 1).

It gives me pleasure to express my obligations to Prof. Gilbert L. Houser, and to Dr. Albert Kuntz for helpful suggestions and for the use of valuable vertebrate sections for comparisons.

THE INTERCALATED DISCS OF HYPERTROPHIED HEART MUSCLE

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TEN FIGURES

In the pathologic heart here considered nature had furnished the experimental condition of overwork leading to exhaustion with complete cessation of function. The specimen is that of a thirty-year-old individual whose important clinical symptom from the present viewpoint was that of failure to respond to any and all cardiac stimulants. The gross specimen was approximately twice the normal size. Microscopically, it is characterized by fragmentation, degeneration of many of the cardiac fibers, and great increase of fibrous tissue. The histologic picture indicates that the beat (weak: 114 per minute) was being maintained by only a relatively small number of greatly hypertrophied fibers. The latter, loaded down by the mass of noncontractile tissue, were finally unable further to sustain the work, and ceased function obviously under condition of over-strain. The matter sought to be elucidated is the effect of these conditions in terms of the intercalated discs, and incidentally the 'stripes' of the fibrils.

The relatively normal (hypertrophied) fibers are broken up into fairly uniform bundles of fibrillae; at the broken ends the ultimate fibrillae appear. These bundles are separated from each other by irregular membranes (Zwischensarkolemme, Heidenhain). The fibrillae present at least four distinct types of structure as illustrated in figures 2, 3, 4 and 5. The polariscope gives no clue as to which band is isotropic and which is anisotropic. Figures 4 and 5 leave no doubt that under certain conditions, the so-called *J* disc is present. Figure 4 gives

absolutely no indication as to which is the *Q* and which the *Z* disc. Apparently here there is no distinction. In figure 5 the only distinction between the darker discs is an alternate deeper staining reaction. In conformity with the usual interpretation the dark disc is the *Z* line. But in figure 2 this band becomes double, and here would be more likely interpreted as *Q*. Figure 3 gives no evidence of the light or *J* disc. Figure 5 probably represents the condition of full contraction, in which the dark disc represents the 'contraction band' of Rollet. Figure 4 more likely represents an intermediate contraction phase between 3 and 5. The seriation of figure 2 is uncertain. It seems clear that much more of detailed physical and chemical knowledge regarding the alteration of striped muscle during contraction, and under the influence of fixing fluids, is needed before the above mentioned pictures can be completely and accurately interpreted. What is of special interest in this connection is the morphologic and tinctorial similarity between the darker 'contraction band' of figure 5 and the intercalated discs of the simpler type, as found in many mammals in foetal (e.g., guinea-pig) and young hearts, and almost exclusively in non-mammalian tissue (fig. 6). This similarity would seem to indicate a close relationship between the intercalated discs and contraction. We[1] have elsewhere interpreted these discs as the result of an irreversible strain condition, i.e., an irreversible contraction. The present study presents further evidence in support of this position, as I shall proceed to show.

In human heart muscle a common type of disc is the 'comb' type. Figure 8 illustrates the more extreme variety. The simpler types of non-human material are readily resolvable into this form; the less wide, apparently compact or granular disc, is simply a 'comb' disc with shorter and in cases more closely packed 'teeth.' We have elsewhere[2] given the arguments for an interpretation of these 'comb' discs in terms of the ultimate fibrillae, the 'teeth' representing local modifications (contractions?) in the ultimate fibrillae. The varieties of this type may

[1] Am. Jour. Anat., vol. 13, no. 2, 1912.

[2] Loc. cit.

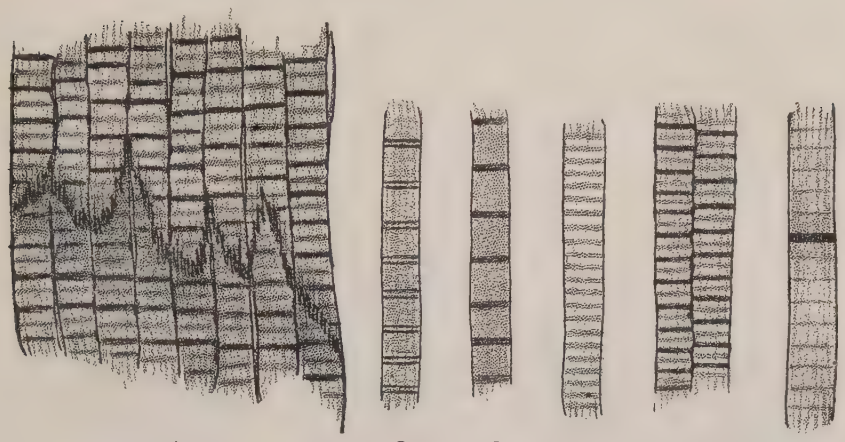

Fig. 1 Typical intercalated disc of hypertrophied cardiac fibers. This muscle fiber is broken up into eight distinct bundles of fibrillae, bounded by distinct membranes. The sarcostyles consist of alternating dark and light segments, with intervening still lighter portions. The darkest segment is probable the contraction band of Rollet; the lighter segment the *Q* disc; and the intervening substance the *J* disc. The several cross-striations of adjacent bundles of fibrillae are frequently at different levels. The immediate region of the discs—sometimes limited to one side—consists of darker-staining sarcoplasm, with darker striations. Leitz 1/16 oil immersion lens, and number 1 ocular.

Fig. 2 A rare type of muscle fiber bundle (of fibrillae), in which the dark stripe appears double, with intervening lighter (*J*?) substance. The alternate much wider segments may represent the *Q* disc. The fiber is apparently in the uncontracted state, but whether at a phase prior or subsequent to that of figure 3 is uncertain.

Fig. 3 Another rare type of bundle. The fiber is evidently relaxed. There is no indication of *J* substance. The darker stripe is not so dark nor compact as the darkest stripe in figures 1 and 5.

Fig. 4 A third rare type of bundle. Here there is simply an alternation of lighter and darker (not as dark as in examples, figs, 1, 2, 3 and 5) discs of approximately equal thickness. These discs may represent the *Q* and *J* substances. But again, as in figures 2 and 3, it is not possible certainly to identify a Krause's membrane. The fiber is apparently in a contracting phase.

Fig. 5 Two adjacent bundles of fibrillae of the usual type in this heart. The fiber is interpreted as in a state of full contraction, the darkest disc representing the contraction band. The micropolariscope gives no indication of alternating isotropic and anisotropic substances. Under crossed Nicols the fibers appear of uniform character, somewhat less dark than the general field.

Fig. 6 Diagram of simplest type of intercalated disc. The darker stripes represent Krause's membranes, the coarser ones thickened by additions of 'anisotropic' substance. The disc is at the level of a dark stripe. It consists of a series of rodlets, modified regions of the fibrillae. This is the only type found below birds, and almost exclusively even in birds; but generally more compact. Note similarity between disc and contraction band, figure 5.

be associated in steps (fig. 7). The 'risers' may possibly be longer modified regions of ultimate fibrillae, rather than one of the membranes (*M* or *Z*) as previously suggested.

A type of disc sometimes met with in mammalian material is the zig-zag type (fig. 9). This consists of a series of very narrow sharp-angled figures both above and below a dark stripe consid-

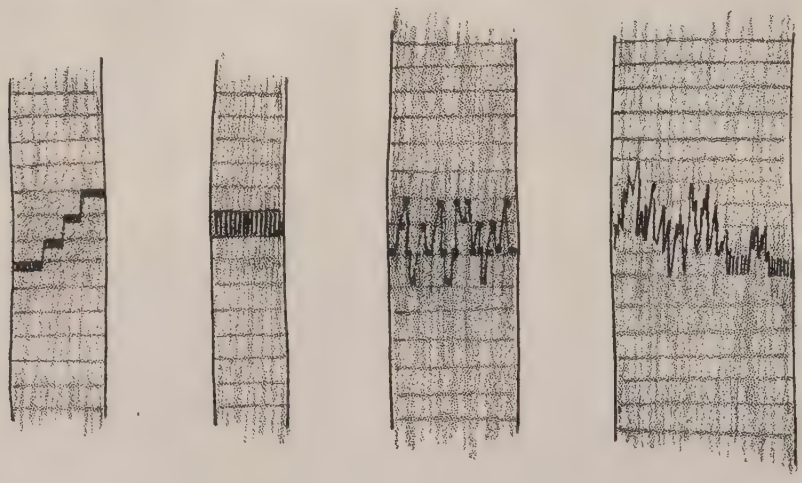

Fig. 7 Diagram of a common condition in mammalian and avian heart fibers. A variable number of discs may be associated to produce step forms. The connecting membrane, 'riser,' may possibly be a modified portion of a fibrilla.

Fig. 8 A fairly common type of disc in normal mammalian heart muscle fibers; derived from the simpler type by elongation of the structural units (rodlets). This and the succeeding type are not generally found in birds.

Fig. 9 Rare type of disc of normal mammalian heart fiber; derived from types 7 and 8, by process of a combination of tensions in the longitudinal and transverse axes.

Fig. 10 Exclusive type of disc of hypertrophied heart muscle fiber; derived from that of figure 9 by exaggeration of the same process. All the types of discs are related to the superficial fibrillae only.

ered as a base line. The crest may be at the level of the first or second succeeding Krause's membrane or may be still farther removed, always ending in a dark-staining granule. The region of the discs is usually characterized by darker-staining sarcoplasm, and darker striations. Fibers containing such discs have a greater diameter than usual. The zig-zag type can be derived

from the 'comb' discs by process of unequal or opposed stresses upon adjacent fibrils, with distortion of a connecting membrane (Krause's membrane). Increase in the transverse diameter of a fiber, combined with a longitudinal tension would produce such result.

In our pathologic heart the only type of disc present in the intact fiber is the zig-zag variety (fig. 1), but very much more complicated than ordinarily seen in mammalian hearts. These discs, as all others, are of little depth, and clearly superficial. The disc is so complicated that it is impossible at present to definitely describe its relation to the several stripes of the fibers. But it clearly represents a modification of the peripheral myofibrillae. If the developmental process of the more complicated type of disc above indicated is correct (and both embryologic and comparative histologic studies give consistent positive evidence on this point) then the condition of extreme complexity here described is clearly the result of a strain condition, to which the discs of hypertrophying fibers are subjected. There is absolutely no evidence here that the discs are cell boundaries (Zimmermann), nor growth regions (Heidenhain). They bear a close relationship to the functional activity of the heart. In their simpler forms they represent local modifications on the fibrillae apparently at the level of Krause's membrane (anisotropic substance?). The more complex types are derived from these by variously associated groups of such modification foci. Their similarity in the simplest form to the contraction band, their more abundant presence in contracted portions of fibers, and in the axis of the muscle mesh, indicate that they are the result of contraction, that is, probably a fixed or irreversible 'contraction band.' The complex type found in the hypertrophied heart is readily conceived of as the result of the unequal strain to which such 'contraction bands' are subjected in overworked and exhausted (hypertrophied) cardiac fibers. Moreover, the discs may have additional physiologic and pathologic significance not yet revealed. The longitudinal splitting of the myofibrillae in hypertrophying fibers is another factor in producing the complexity of this type of disc.

The fact that hypertrophied heart muscle is characterized by a specific type of disc which can be derived from the common type of normal fibers by process of the action of simple mechanical factors of transverse and longitudinal tension—such as obviously prevail in hypertrophying fibers—disposes of any hypothesis of growth phenomena as related to intercalated discs. Given the simple common 'comb' type, the zig-zag type is readily derived without assuming any growth activity. All the appearances suggest that the disc is an inert structure subsequent to its origin, modified only by mechanical factors consequent upon varying functional conditions.[3] The similarity between the simpler discs and the 'contraction band' suggests the method of origin, i.e., by contraction or strain. In view of Meigs'[4] recent work on the wing muscle of the fly in which material in the contracted state he was unable to distinguish a differentiation of isotropic and anisotropic substances, the fiber illustrated in figure 1—in which also there seemed to be no delimitation of such substances—is probably in a contracted state. Accordingly, the inference that the darkest disc is a 'contraction band' seems the more legitimate; and the hypothesis of the origin of intercalated discs as coincident with extreme contraction is rendered still more plausible.

[3] I find that Dr. A. Dietrich in a monograph, "Die Elemente des Herzmuskels" (Jena, 1910), which came to hand only after my paper was in press, expresses an essentially similar view respecting the origin of a characteristic form of intercalated disc in hypertrophied heart muscle. It is outside the scope of the present contribution to make the critical and extensive references which this important work deserves, and which it properly should have received in my recent papers on intercalated discs. It must suffice simply to note that Dietrich does not recognise any connection between the presence of discs and contraction phenomena; whereas certain facts, already stated, lead me to regard the discs as irreversible contraction bands, or strain effects, subsequently variously modified.

[4] Zeitschr. f. Allgem. Phys., Bd. 8, no. 1, 1908.

A CASE OF ACCIDENTAL IMPREGNATION OF CELLS IN THE BRAIN OF A HUMAN EMBRYO OF FOUR MONTHS

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FOUR FIGURES

The heads of twin embryos of about four months were fixed in Zenker's fluid. They were afterwards kept in the same jar, containing about 82 per cent alcohol, for several years. The brains were removed and stained in toto with paracarmin. The first of these to be sectioned took the stain very well and showed no sign of precipitate. The second was sectioned soon after this and it was noticed that although the stain was equally as good as in the first specimen, there was a considerable amount of precipitate throughout the series. Some of this precipitated material seemed to be irregularly massed in various parts of the brain, but much of it was well organized giving Golgi-like pictures of blood vessels, cells and fibers. Most of the impregnated cells were near the brain cavities and located in the ependymal layer. Most of these cells were bipolar with long processes extending out towards the surfaces of the brain (figs. 1, 2 and 3). In the more central portions of the sections there were a few masses, appearing like multipolar cells with many fine fibers, which were also impregnated. One of these masses shown in figure 3, was evidently a neuroglia cell, but those shown in figure 4 were probably true nerve cells.

The cells were for the most part well impregnated in special regions of the ependyma and chiefly confined to that layer in all parts of the brain. Almost no cells were found in the cerebrum or cerebellum, while the ependyma of the thalamus, optic lobes and medulla had great numbers of cells, chiefly of a bipolar form, with long fibers. Perhaps some of these cells were stages of neuroblasts, but many if not most of them were evidently spongioblasts and were found with their cell bodies showing at all levels of the ependyma (figs. 2 and 3). In some places as in figure 3, some of these cells show beyond the ependymal layer.

The impregnation was very perfect in many cases. The size of some cells and especially some fibers was evidently exaggerated, judging from the adjoining unimpregnated but carmine stained nuclei (fig. 1).

In some of the impregnated cells at the borders of the ventricles, part of the material was seen to project into the brain cavity (fig. 3). This may indicate the position if not the form of cilia.

The precipitate obtained in cells, fibers and blood vessels, may simply be the deposit so often seen after mercuric chloride fixation; however the specimen described seems to have this substance organized as one

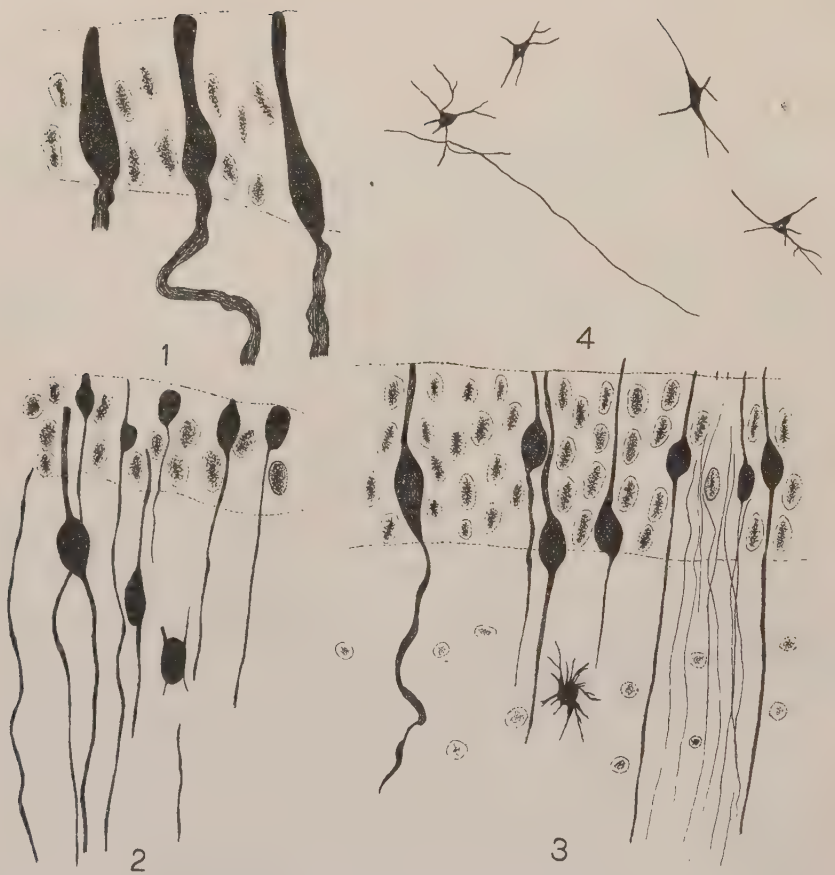

Fig. 1 Camera lucida sketch of cells from the olfactory region of a four-month human embryo. The outline of the ependyma is shown with impregnated cells. The nuclei of unimpregnated cells are also shown. × 550.

Fig. 2 Camera lucida sketch from a portion of the inner surface of the brain near the hippocampus. × 550.

Fig. 3 Camera lucida sketch of cells, fibers, ependymal nuclei and deeper nuclei from the thalamus. × 550.

Fig. 4 Camera lucida drawing of several cells from the deeper parts of the medulla. × 80.

would expect to find it in material prepared by Golgi's bichromate and sublimate method.[1] Like results are given by the Cox modification of this method when both salts are used at the same time.[2]

[1] Un nuovo processo di tecnica microscopica. Rendic. R. Inst. Lombardo, tome 12, 1879.

[2] Impragnation des centralen Nervensystems mit Quecksilbersalzen. Arch. Mikr. Anat. Bd. 37, 1891.

THE USE OF THE GRAVER'S POINT IN DISSECTIONS

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Last year I had occasion to make some preparations of the inner ear to demonstrate the labyrinth and tympanic cavity and it occurred to me to test the efficiency of the engraver's points as instruments for this kind of dissection. The ease with which excellent results could be obtained by the use of this instrument immediately suggested its practicability in the hands of students for similar purposes. Accordingly it was made a requirement this year that each student's dissecting set should be supplied with a graver's point.

It has been my experience that with the more usual methods of employing a mallet and chisel for these fine dissections the student, with uncertain technique and judgment, is quite prone to ruin the whole specimen at a single blow. With these engraving instruments, however, it was found to be a very simple and easy matter to guide the students' work and lead them to obtain very perfect results. The results of this year's work with the instrument were decidedly satisfactory, notwithstanding the fact that in a class of ninety there were men quite widely differing in the matter of previous training and endowment with natural talents.

In the dissection of the inner ear, the method of work which was outlined for the students may be briefly stated in the following manner. The student is first required to make himself as well acquainted as possible with the anatomy of the ear by studying the Auzoux models with the aid of his textbook. When this is accomplished he begins the actual dissection of the ear on his cadaver.

The periosteal layer of the dura is removed from the petrous part of the temporal bone, using care not to tear away the great superficial petrosal nerve. With the graver's point a groove is cut transversely to the long axis of the petrous bone and approximately over the course of the superior semicircular canal. The position of this canal is about 8 mm. lateral to the internal acoustic meatus and is indicated by the more or less indistinct arcuate eminence.

This groove is deepened and widened by successive cuts until it opens into the diploë. This diploë occasionally proved confusing to some students as its appearance often suggests a canal. Complete removal of the diploë exposes a rounded ridge of hard bone which gives accurately the position of the superior canal. A V-shaped groove cut along the course of this ridge and gradually deepened, will soon expose the small

lumen of the canal. Inserting a bristle into the lumen, it is easily opened up to its ampulla in front and backwards to the crus commune behind.

It is next most convenient to open the posterior semicircular canal, starting from the crus commune and cutting away the bone forming the posterior wall of the canal until its ampulla is reached inferiorly.

A considerable amount of compact and cancellous bone which lies lateral to the superior canal, must now be carefully cut away until the upper level of the lateral semicircular canal is reached. To open this canal the bone forming its upper wall is cut away, thus laying it open from its ampulla back to the crus simplex. This leaves the facial nerve untouched and uninjured in its position directly beneath this canal.

The semicircular canals having been demonstrated, exposure of the facial nerve is commenced by cutting away the roof of the internal acoustic meatus. The nerve is thus traced as far as the geniculate ganglion and the connection of the great superficial petrosal nerve with the latter is easily demonstrated. The further course of the facial nerve beneath the lateral semicircular canal is now traced by cutting away the lateral wall of the facial canal.

By turning aside the facial nerve from its position in the internal acoustic meatus, the cochlear nerve may be seen bending forwards into the tractus spiralis foraminosus. In order to expose the cochlea the bone that lies beneath the knee of the facial nerve may be cut away by using the edge of the graver instead of its point. The basal coil of the cochlea becomes exposed first and its connection with the vestibule may be demonstrated by the insertion of a bristle. The smaller coils and modiolus may be completely exposed by cutting deeper into the bone in front and medial to the basal coil.

When these more delicate parts of the internal ear have been worked out the tegmen tympani is next cut away, using care not to disturb the ossicles. The chorda tympani is first identified in its position medial to the neck of the malleus and then the bone is completely cut away from it as it passes from the front margin of the tympanic membrane into the petrotympanic fissure.

By the removal of a considerable amount of bone posterior and lateral to the horizontal semicircular canal, the remaining portion of the facial nerve is exposed in its downward bend towards the stylo-mastoid foramen. The exposure of the first part of the chorda tympani may now be completed by cutting open its canaliculus backwards from the posterior margin of the tympanic membrane to the point where it leaves the facial nerve.

After the chorda tympani has been completely exposed the structure and relations of the tympanic cavity may be studied. The roof of the cavity and of the cochleariform canal is more completely cut away and the incus carefully removed so as to allow a better view of the cavity. The M. tensor tympani and the auditive tube are also easily traced out.

Successful results with the use of the graver's points are best obtained by holding and using the instruments in the same manner as does the engraver. The handle of the instrument is held securely against the

palm of the hand and the point pushed forward in the desired course, making, by successive cuts, a groove in the bone. Attempts to cut too deeply, or chipping dissections, may lead one into difficulties or accidents. To prevent slipping and to guide the instrument more accurately it is necessary to brace the hand by holding a finger or thumb against some part of the specimen. It is essential, for good results, that the instrument shall not be too long and so it is often desirable to break off a piece from the shank. Instead of the regulation handle a champagne cork is often found an excellent and convenient substitute.

The large variety of shapes in which graver's points may be obtained suggests a great many possibilities for fine dissections in which there are delicate nerves or other structures. The diamond-point graver appears to be the best form, however, for general purposes.

The practical value of this instrument, as adapted to anatomical dissections, may be summed up as follows:

1. It makes possible and practicable a perfect dissection of the middle and inner ear and of similar minute structures. Any of the delicate dissections in Spalteholz's Atlas may be easily reproduced.

2. The student's dissection of the middle and inner ear can be easily and perfectly controlled by the instructor and accidents, which might spoil the specimen, are entirely avoidable.

3. The best and most practical method for the student to learn the anatomy of the internal ear, and for the orientation of it in relation to the temporal bone and to the head in general, is for him to study first an accurate model and then do a perfect and careful dissection upon the cadaver. Such a dissection is possible in every detail with the use of the graver's point.

4. With these instruments a perfect dissection of any of the minute structures in bone is quite an easy matter—such as the terminal ramifications of the dental nerves, the small branches of the trigeminal, facial, glossopharyngeal or vagus nerves and their ganglia; or the dissection of small arteries and muscles of the ear or other parts of the head.

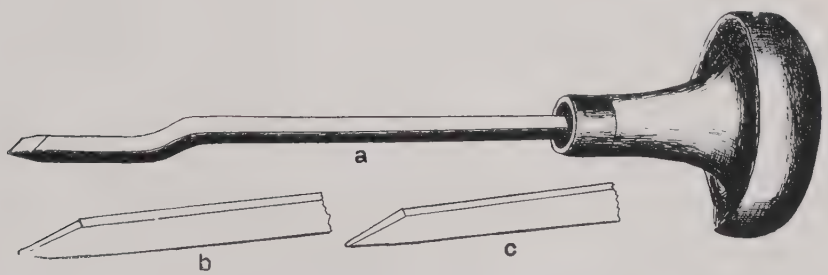

Fig. 1 *a*, The diamond point graver; *b*, a point with rounded end for cutting narrow grooves; *c*, a point having a narrow, square end.

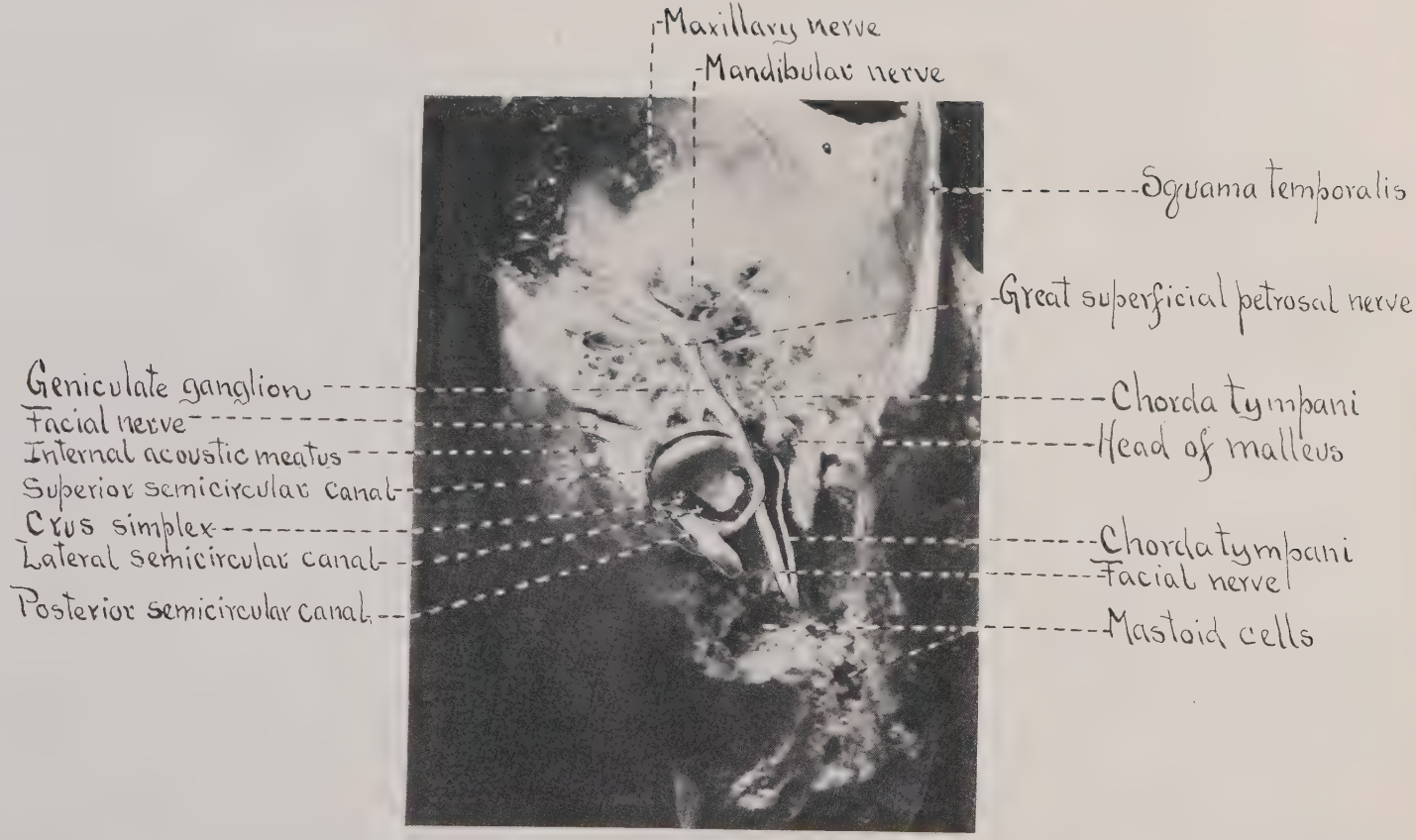

Fig. 2 A dissection of the ear made by using the diamond-point graver. Dissection by Mr. C. N. Kavanaugh. The nerves are whitened and slightly accentuated. Photograph. × 1.36.

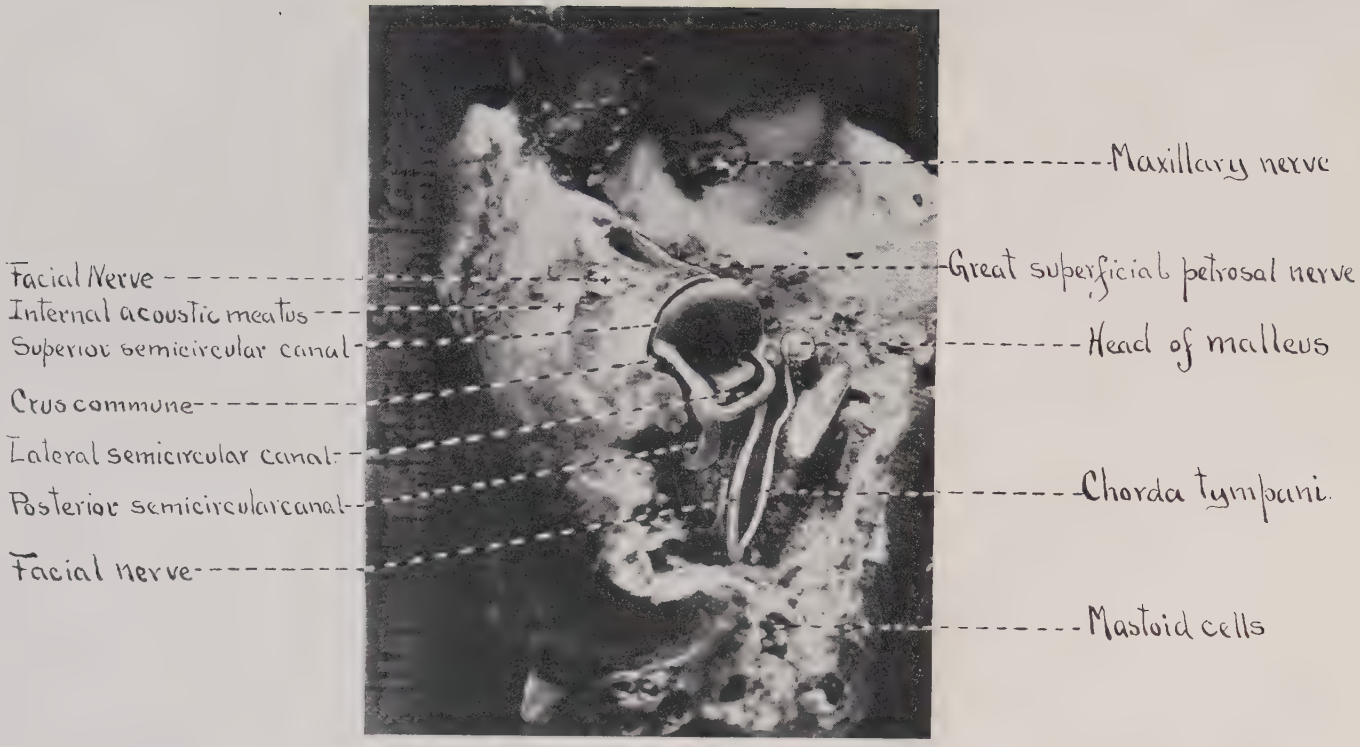

Fig. 3 Showing the semicircular canals (which were blackened in the specimen in order to make them appear distinctly in the photograph). × 1.52.

BOOKS RECEIVED

A TEXT-BOOK OF HUMAN PHYSIOLOGY, including a section on Physiologic Apparatus, Albert P. Brubaker, fourth edition, revised and enlarged, with 1 colored plate and 377 illustrations, 736 pages including Index, 1912, $3.00. P. Blakiston's Son and Company, 1012 Walnut S reet, Philadelphia.

COMPARATIVE ANATOMY OF VERTEBRATES, J. S. Kingsley, with 346 illustrations, 401 pages including Index, 1912, $2.25. P. Blakiston's Son and Company, 1012 Walnut Street, Philadelphia.

PRACTICAL ANATOMY, an exposition of the facts of gross anatomy from the topographical standpoint and a guide to the dissection of the human body, John C. Heisler, with 366 illustrations, of which 225 are in color, 790 pages including Index, 1912, $4.50. J. B. Lippincott Company, Philadelphia and London.

A SIMPLE TECHNIQUE FOR THE REMOVAL OF THE HYALOID MEMBRANE WITH CONTENTS AND ATTACHMENTS INTACT

FREDERIC A. WOLL

From the College of the City of New York

ONE FIGURE

In eye dissections it is quite customary, in giving directions for dissections merely to mention the hyaloid membrane and its relations with other parts of the eye. Rarely is there any attempt made to isolate it. Often, too, the retina is mistaken for the hyaloid and the retina then wrongly demonstrated as being attached to the choroid. If the following simple technique is carefully observed the membrane, with all its connections, can be easily separated from other parts of the eye. Opportunity for thorough study and observation will then be made extremely easy.

Procure the eye of either a sheep or of a bullock. Instead of following the usual procedure of hardening in any one of the several solutions used for the purpose of toughening the ocular tissues, place the eye in a cool place and permit it to collapse a trifle. Usually a day or two is sufficient to accomplish this. With a pair of dissecting forceps pinch up the sclerotic about 5 mm. anterior to the equator. With a pair of small, fine pointed scissors, make an incision. Next hold the eye in the left hand without exerting any pressure. Insert the point of the scissors into the incision made and cut. Be careful to keep the point of the scissors close to the sclerotic or an untimely puncturing of the choroid will occur. Continue the cutting on a line parallel to the equatorial meridian and about 5 mm. anterior to it until about half the sclerotic has been separated. In cutting always move the scissors point forward with a slight oscillating lateral movement. Also, while doing this, partly suspend the eye-ball from the point of the scissors. Doing these things will tend to loosen the choroid from the sclerotic and prevent the puncturing of the former mentioned membrane too soon. Now apply pressure in such a manner that the lips of the cut sclerotic will gap. Into this put the point of the scissors and very carefully separate the choroid and the retina. If the choroid alone is separated the retina will show milky white or yellowish white underneath. The retina must also be separated. Care must be taken not to go deeper than the retina or the hyaloid may be damaged. Continue the cutting of the choroid

and the retina for about a distance of 20 mm. Apply enough pressure occasionally so that the vitreous will be forced upward above the choroid and the retina. This will show whether any strands of the two membranes have been left uncut. If the separation is complete for the distance specified above, invert the eye-ball, squeeze and shake gently over some receptacle, such as a Stender dish, three-fourths filled with a $2\frac{1}{2}$ or 5 per cent solution of formaldehyde, and the hyaloid membrane containing the vitreous, its attachments, suspensory ligament to the lens capsule, and lens will drop out intact. It is unwise to use alcohol

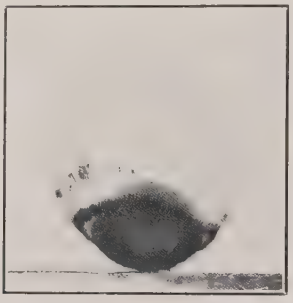

Fig. 1. Photograph of dissected hyaloid membrane, with pigmented indentations of the ciliary processes, the suspensory ligament, and the crystalline lens in its capsule.

as a preservative since it produces an almost immediate opaqueness and hardness which spoils the specimen for further study.

This description may give the mpression that the dissection is a lengthy one; however, it can be done by an expert in two or three minutes. By a beginner in five or six minutes.

For purposes of demonstration or study the specimen should be placed in a small bottle or vial containing a 5 per cent solution of formaldehyde. It can then be examined macroscopically, or with hand lens or microscope.

ON THE RELATION OF THE CHORDA DORSALIS TO THE ANLAGE OF THE PHARYNGEAL BURSA OR MEDIAN PHARYNGEAL RECESS

G. CARL HUBER

From The Wistar Institute of Anatomy and Biology

SEVENTEEN FIGURES

The pharyngeal bursa, first described by A. F. J. C. Mayer in 1840, constitutes a more or less clearly defined depression found in the pharyngeal vault in connection with the lower part of the pharyngeal tonsil usually having an upward and backward direction. There is a question as to the constancy of the existence of this structure in the adult, but its presence in early childhood is very generally admitted. There exists in the literature a diversity of opinion as to the origin and nature of the pharyngeal bursa. Luschka regarded this bursa as the remains of the oral hypophysial duct, basing his deduction on evidence gained from a human monstrosity, which was examined, however, only imperfectly. Through the more careful investigations of Dursy this view was shown to be incorrect. Schwabach regards what is known as the pharyngeal bursa as merely a crypt connected with the formation of the pharyngeal tonsil. Killian regards this bursa as a structure *sui generis*. Robert Meyer regards this bursa as found in the adult as identical with the Seessel's pocket described for early embryonic stages. A number of observers have called attention to the fact that there exists a definite relation between the notochord and the pharyngeal epithelium in the region where the pharyngeal bursa occurs, and ascribe a causal relation to this union of notochord and the pharyngeal entoderm as concerns the anlage of the pharyngeal bursa, and it is to this phase of the question that the following more careful review of the literature and my own contribution is directed. For a more comprehensive

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review of the literature the reader is referred to the well known contribution of Killian, and to the brief but excellent exposition of the various views held concerning the origin and nature of the pharyngeal bursa as given by Oppel.

Froriep in his account of the development of the head portion of the chorda dorsalis in human embryos describes and figures the relation of this structure in a human embryo of 3.8 cm., in which the notochord of the retropharyngeal region, that portion of the notochord which in the human embryo lies ventral to the spheno-occipital anlage, presented four enlargements. One of these is quite prominent, and is situated 1 mm. in front of the region where the notochord leaves the ventral surface of the occipital cartilage. In this region there was observed an opening in the chordal sheath through which the chordal epithelium was directly connected with looped chords of chordal cells, which came in close relation to a well developed pharyngeal bursa. Whether the chordal epithelium was blended with the pharyngeal entoderm was difficult to ascertain. Concerning this Froriep expressed himself as follows: "Man hat sogar bei gewisser Einstellung den Eindruck, als ob die Zellen des Epithels und der Chorda sich unmittelbar berührten, jedoch nicht in so überzeugender Weise, dass ich diese Stelle in die Zeichnung aufzunehmen gewagt hätte." Of the six human embryos investigated by Froriep, these varying in length to from 1.7 cm. to 8.8 cm., only one presented a distinct pharyngeal bursa. In discussing his results, this observer draws especial attention to the observation above referred to, as may be seen from the following statements: "Eine entwicklungsgeschichtliche Aktion scheint in dessen dem Kopftheil der Chorda, wenigstens bisweilen, zuzukommen, dieselbe erstreckt sich aber nicht auf den Schädel, sondern auf die Schleimhaut des Pharynx; es ist die Bildung der sogen. Bursa pharyngea." . . . . "Das in Fig. 3*a* abgebildete Präparat zeigt die Spitze einer embryonalen Bursa pharyngea eingesenkt in einen Haufen von Zellensträngen, welche der Retropharyngeal-Chorda angehören. Ein wirklicher Zusammenhang von Epithelzellen mit Zellen der Chorda ist zwar mit Evidenz nicht nachzuweisen, es drängt sich aber bei der Untersuchung doch die Ueberzeugung auf, dass diese fast zur Berührung führende Nebeneinanderlagerung der beiden Gewebs-individualitäten keine gleichgültige sein kann. Die Wahrscheinlichkeit spricht dafür, dass in den Fällen, wo eine retropharyngeale Chordaanhäufung bis an das Epithel des Schlundkopfs vordringt, eine Beziehung zwischen beiden sich herstellt, welche die berührte Stelle der Schleimhaut oder ihres Epithels bei einer später etwa folgenden Abdrängung der Schlundwand von der Schädelbasis an dieser festhält, and so jene trichterförmige Ausstülpung, die sogenannte Bursa pharyngea, zur Entstehung bringt." Some years later Nebelthau confirmed this observation of Froriep, as may be seen from the following quotation: "Ebenfalls scheint auch die Vermutung Frorieps, dass der Wirbelsaite die entwickelungsgeschichtliche Aktion zukomme, durch eine enge Beziehung zum

Rachenepithel, eine gewisse Fixierung eines Teils seiner Zellen, jene trichterförmige Ausstülpung, die sog. Bursa pharyngea zur Entstehung zu bringen, durch den Befund meines Präparates eine Stütze zu finden." Killian, whose observation on the bursa and tonsilla pharyngea have been frequently quoted, studied this region in 65 human embryos, 3 newborn and 2 children, in all, 70 cases. The embryos and fetuses studied varied in ages from three months to eight months. It is to be noted that the younger stages are lacking. In 28 of the 70 cases studied (40 per cent), no bursa was present. The 42 cases in which a bursa was present he divides into such as presented a well developed bursa, 14 in all, and such as presented a more or less pronounced groove or funnel-shaped depression, 26 in all. The region in question was studied macroscopically in all cases; 20 of the cases, varying in age from four to eight months, were sectioned and studied microscopically. Killian recognized the fact that all of the embryos studied, including those sectioned, were too old to present suggestions as to the anlage and development of the bursa, neither does he find evidence for this in the well known work of His: "Zur Anatomie menschlicher Embryonen." For data concerning the earlier stages he mentions the observations of Froriep, above referred to. Killian reviews critically Froriep's theory that a pharyngeal bursa develops in case a retropharyngeal accumulation of chorda cells reaches the pharyngeal epithelium, and comes to the conclusion that this theory possesses the fault that it is based on a single observation. Furthermore, since the bursa always develops in a definite region, namely—just in front of the uppermost fibers of the constrictor pharyngis super. which comes in close contact with the lower or posterior wall of the bursa as it lies between the heads of the musculi long. capitis, both of which relations may be regarded as characteristic—while, as has been shown by Froriep, several accumulations of chorda cells which approach the pharyngeal epithelium are found in the retropharyngeal region, and there is no reason why a bursa should not develop anterior to its normal position if close relation of chorda cells and pharyngeal epithelium are to be brought into causal relation as concerns the anlage of the pharyngeal bursa. Killian also dismisses the lig. occipito-phar. as a factor in the development of the bursa and the fact that in a number of his cases the oral hypophysial duct and the bursa were both present and separated by appreciable distances, excludes the view that persistence of the hypophysial duct and the cranio-pharyngeal canal may be regarded as the anlage of the pharyngeal bursa. Killian, therefore, regards the bursa as a 'true epithelial outgrowth,' as may be seen from the following statement, summarizing his discussion of the anlage of this structure: "Ich betrachte also die Bursa pharyngea embryonalis als eine wahre Ausstülpung der Rachenschleimhaut nach hinten und oben gegen das Hinterhauptsbein, wobei speciell im Epithel das formative Princip zu suchen ist. Sie verdankt nicht mechanischen Einwirkungen, sondern einem aktiven Bildungsvorgange ihre Entstehung." Killian presents further very extended comparative anatomic studies, which it would be impossible to cite here in detail. It may be stated, however, that his results

in the main were negative, in that no bursa pharyngea was present in the great majority of the types investigated including representation from vertebrate classes ranging from primates to amphibia. A true pharyngeal bursa as found in man was observed only in Arctomys marmota, although the pig, deer and bear presented structures which have been regarded as pharyngeal bursae, and may be homologues of this structure as found in man. Schwabach treats of the median pharyngeal bursa in two contributions. In the first of these he discusses observations made on 52 heads, of which 28 were of children ranging in age from one day to three years; to these may be added a human fetus of six months. As a result of these studies he is led to believe that the pharyngeal bursa of Luschka represents merely the end of the median depression found in the pharyngeal tonsil, therefore, is not to be considered an anatomic entity, but a part of the pharyngeal tonsil. In the second contribution Schwabach treats of the development of the pharyngeal tonsil. In all, 45 embryos were studied, of these 43 presented a crown-rump length of 3.1 cm. or more. In these the vault of the pharynx was exposed by removal of the lower jaw and other necessary parts. The preparations thus obtained were then examined macroscopically; certain of them were then cut in sagittal or frontal sections. Two other embryos of 1.6 cm. and 2.8 cm. crown-rump measurement were cut in sagittal sections. In embryos of less than 6 cm. length no evidence of any depression or infolding of the pharyngeal vault was observed. In embryos ranging from 6 cm. to 7 cm. in crown-rump length there was noted in the median line at the transition of the fornix to the posterior pharyngeal wall a shallow depression or groove which was regarded as the anlage of the pharyngeal tonsil. This depression is said to have reached its maximum in embryos of from 9 cm. to 10 cm. crown-rump length, after which period of development the depression again became shallower, as the adenoid tissue of the pharyngeal tonsil became evident. Kölliker in his brief discussion of the head chorda makes no mention of its bearing any relation to the pharyngeal bursa, which latter structure is not considered in this connection. In a brief note, entitled "The Notochord of the Head in Human Embryos of the Third to the Twelfth Week," Mrs. Gage expresses very clearly and correctly the relations existing between the head chorda and the pharyngeal entoderm, as the following abstract may show: "In a human specimen of sixty days the relations of the notochord to the cartilaginous base of the skull and the epithelium of the mouth are clear. On emerging from the axis it forms a knotted protuberance dorsal of the base of the skull, passes diagonally through it to a pocket from the roof of the mouth, thence cephalad to come in contact with two other mouth pockets, thence diagonally dorsal through the base of the skull, again forming a knot and turning sharply ventrad, ending near the hypophysis, but within the cartilage." The same relations exist in a specimen of forty-eight days, and at thirty-six days where a condensed mesoderm foreshadows the skull, and at twenty-eight and twenty-one days where the notochord lies directly in contact with the epithelium of the roof of the mouth, thus showing the beginning of the history. Mrs. Gage further reports briefly on a comparative study of

the head chorda of the pig, sheep, calf, mouse, cat, chick, amblystoma, frog, shark and lamprey, and shows that the notochord after the earliest stages is usually completely separated from the roof of the mouth. In the pig, however, about 20 per cent were similar to man, being in contact with mouth pockets. Mrs. Gage has very kindly sent me a number of photomicrographs of the head region of a number of human embryos of the Cornell series, cut in very favorable sagittal sections, which show very clearly the relations of the notochord as described by her; for this I desire to express my sincere thanks.

Giuseppe Levi in his study of the development of the cartilaginous primordial cranium, considers the relations of the head chorda in the human embryos at his disposal. In one of the embryos studied by him, his embryo *B*, greatest length 14 mm., he states that the notochord after reaching the ventral surface of the basal plate and after coursing in the perichondrium for a short distance, passes into the retropharyngeal connective tissue; "wo sie mit dem Epithele der Bursa pharyngea einen innigen Zusammenhang hat." In the other embryos studied no such relations were observed. Göppert in his discussion of the development of the oral cavity and its organs in the 'Hertwig Handbuch,' gives brief consideration to the development of the Bursa pharyngea, quoting largely from the studies of Killian, previously considered, and accepting his views. Gaupp in his chapter on the development of the head skeleton in the 'Hertwig Handbuch' considers the relations of the head chorda to the basal plate. The relations as found in rabbit and human embryos are chiefly discussed, for the latter the description given is based mainly on the observations of Froriep, previously noted; no mention is made of the relations of the head chorda to the pharyngeal epithelium. Williams in his study of the development of the notochord, especially in that portion which deals with the relations of the notochord to chordoma, considers the head chorda in a 32 mm. human embryo (H.E.C., No. 292) giving an illustration, his figure 20. This figure shows the head chorda as incorporated in the cartilaginous basal plate, and lying near the surface of the cartilage at four points. "It is near the upper surface, in the hypophysial fossa, a short distance behind the fossa, and near the foramen magnum and near the lower surface at a point midway between the hypophysial fossa and the foramen magnum." This, it is stated, is the normal course of the notochord in the skull of human embryos, its curve being due to the fact that the notochord remains attached to the epithelium of the vault of the pharynx longer than elsewhere, the mesenchyma which grows in between the base of the brain and the pharynx collects, therefore, above the middle portion of the notochord. Williams further states that this middle section of the notochord bears a variable number of kinks, short branches and thickenings which he often finds involving the pharyngeal epithelium, which is here thickened and often invaginated. Special mention of the relation of the head chorda to the epithelium of the pharyngeal bursa, if this was noted, is not given. Meyer, in a brief note, draws especial attention to the relation of the chorda to the development of the median pharyngeal bursa or recess in human embryos. A concise statement of his findings in human

embryos varying from 2.5 mm. to 40 mm. in length is given, in all of which he shows a more or less close relation of the chorda to the epithelium of the pharyngeal bursa. His results are summarized as follows: "Aus meinen Befunden an jüngeren menschlichen Embryonen geht hervor, dass die Bursa pharyngea unabhängig von der Rathkeschen Tasche im 2. Monate bei ca. 14–28 mm. Scheitelsteisslänge ungefähr unter 5 Fällen einmal zur Ausbildung kommt und stets mit der Chorda im Zusammenhange steht. Ich habe daraus die Anschauung gewonnen, dass als direkte Ursache der inkonstanten Bursabildung beim Embryo eine Persistenz der ursprünglichen Verbindung zwischen Chorda and Rachenentoderm anzuschuldigen ist, also eine mangelhafte Lösung zwischen beiden." In this note Meyer cites the observations of Grünwald on sheep embryos, to which I shall refer later, who records that the anterior end of the chorda remains in contact with the pharyngeal membrane and ultimately with the epithelium of Seessel's pocket, and further, that Froriep had previously called attention to the contact of the anterior end of the chorda with the epithelium of Seessel's pocket in chick embryos, and that Staderini had shown similar relations for rabbit and sheep embryos. This leads Meyer to conclude, that "Der Umstand, dass die Bursa pharyngea der Erwachsenen ebenfalls an der Schädelbasis fest adhärent gefunden wird, weil sie die Fibrocartilago basilaris durchbohrt, erlaubt den Schluss, dass die Bursa pharyngea media der Erwachsenen mit der bei Embryonen beschreibenen, also der Seesselschen Tasche identisch ist." Linck in an extensive contribution on the chorda dorsalis in the head and neck regions of human embryos, in which embryos varying in length from 2 cm. to 25 cm., head to foot measurement, with leg extended, are very fully considered, deals fully with the relations of the head chorda to the pharyngeal bursa. This relation is further considered by Linck in an especial contribution dealing with the genesis of the embryonic pharyngeal bursa, a contribution which must be regarded as the most comprehensive and accurate of all dealing with this subject. In nine of the sixteen embryos studied there was present an embryonic pharyngeal bursa. In only one of the nine embryos considered as presenting this bursa, however, is there direct contact of chorda tissue and pharyngeal epithelium, and in this embryo, No. 1, 2 cm. length, this contact must be assumed, since the pharyngeal epithelium was in large part wanting, owing to maceration. In the other eight embryos presenting this bursa, which is figured for nearly all of the preparations in one or the other of his two publications, there were observed chordal remains in more or less close relations to the blind end of the pharyngeal bursa enclosed within a more or less clearly recognized strand of developing connective tissue extending between the region of exit of the chorda from the basal plate to the blind end of the pharyngeal bursa. Linck recognizes as causative factors in the development of the embryonic pharyngeal bursa the attachment of the chorda to the pharyngeal epithelium in a region corresponding to about the middle of the basal plate in early embryonic stages, and tension on the chorda resulting by reason of a caudal elongation of the basal plate in further growth,

this accompanied by a thickening of the retropharyngeal connective tissue as growth proceeds, as may be seen from the following extract taken from his summary: "Diese stets an einer typischen Stelle, ursprünglich etwa über der Mitte des Grundknorpels, gelegene Verbindung zwischen Chorda dorsalis und Rachenoberfläche bringt im Verein mit dem Dickenwachstum der Rachenhaut und dem ungleichmässigen Langenwachstum des os basilare einen Mechanismus zustande,welcher bewirkt, dass der aufsteigende Chordaschenkel auf die Schleimhautoberfläche einen elastischen Zug ausübt und eine Kaudalwärts gerichtete Epitheleinstülpung, die Bursa pharyngea embryonalis, hervorbringen kann." I shall refer again to the contributions of Linck after presenting my own observations.

The material on which my own observations were made includes human embryos from Professor Mall's collection, mainly younger stages, and from my own collection. Only embryos cut in sagittal sections and of at least fairly good preservation are included. In all, 15 embryos, varying in crown-rump or-breech length, from 6.6 mm. to 145 mm. were especially studied, and, as will be observed, the embryos selected form a fairly complete series between the lengths given. Of nearly all there is given an outline drawing of a mid-plane sagittal section of the head, particular attention being paid to the head chorda and its relation to the pharyngeal epithelium, as also the pharyngeal bursa whenever this was present. These figures were made by graphic reconstruction. The reconstruction was, however, not extended in all of the figures, so as to attain a mid-plane sagittal section of the nervous system, nor the tongue region. The detail figures, 1*a* to 14*a*, were drawn by aid of camera lucida at a magnification of 300 diameters, reduced in reproduction so as to give a magnification of 100 diameters, and for the greater part were drawn from a single section. A few of these figures are combined from the drawing of two or three sections. Each detail figure includes a special region where the notochord comes in contact with the pharyngeal entoderm, for the younger stages, a region which I regard as the seat of the pharyngeal bursa; for the older stages, the region of the pharyngeal bursa and surrounding retropharyngeal tissue is given. The data here given were presented by way of demonstration at the Boston meeting of the American Association of Anatomists, December, 1909, and again in an

address with projection demonstrations given at the College of Physicians and Surgeons of Philadelphia in May, 1911. The present presentation seems warranted by reason of the fact that the series of embryos on which this study is based, includes the essential younger stages lacking in the contribution of Froriep and Linck, and I hope to make clear that these younger stages show that the pharyngeal bursa bears no relation to Seessel's pocket as is postulated by Meyer. I may now proceed with a brief consideration of the material at my command.

Embryo A, No. 371 of the Mall collection, 6.6 mm. length. Figure 1 and figure 1*a*. There is present a large and well fixed notochord which after it passes through the denser mesenchyme of the vertebral anlagen, bends slightly ventrally beneath the denser mesenchyme, the anlage of the basal plate. In the region where the notochord bends ventrally to pass beneath the anlage of the basal plate it presents a slight enlargement and comes in contact with the pharyngeal epithelium. The notochord touches the epithelium again slightly cephalad to this area, and again just before it bends dorsally near its cephalic end. Otherwise the notochord is completely separated from the pharyngeal epithelium by one or two rows of nuclei of the mesenchyme. The extreme cephalic end of the notochord presents a slightly wavy and spiral course and bends ventrally to reach the pharyngeal epithelium just caudal to Rathke's pouch, as is shown in figure 1. The extreme anterior end of the notochord is somewhat difficult to make out clearly, and it is not possible, therefore, to state with certainty that the tip end is actually in contact with the pharyngeal epithelium. The region where the notochord first touches the pharyngeal epithelium as one passes from the caudal to the cephalic portion of the pharyngeal vault, just after the chorda bends ventrally to pass into the retropharyngeal region, near the right upper end of figure 1*a*, represents the place of development of the pharyngeal bursa. This region of contact of notochord with pharyngeal epithelium is constant and characteristic for the younger stages. It is always caudal to the thyroglossal pit in the tongue, lower left side of figure 1*a*, though the distance varies somewhat, due no doubt to the extent of flexion of the head. The place where the cephalic end of the notochord terminates in this embryo is the region of Seessel's pocket, though a distinct pocket is not observed in this preparation, and it is a question whether this pocket exists in the human embryo. It has been shown that the notochord ends in Seessel's pocket in chick, rabbit and sheep embryos in

early stages of development. The same seems, therefore, true in the human embryo. The fact that in Embryo *A*, of this series, the area of contact of notochord and pharyngeal epithelium, indicating the region of development of the pharyngeal bursa and the extreme anterior point of contact in the region of Seessel's pocket may both be made out and separated by practically the whole length of the pharyngeal vault, would seem to me to suffice to controvert the contention of Meyer that the pharyngeal bursa develops from Seessel's pocket.

Embryo B, No. 221, Mall collection, 7.5 mm. Figure 2 and figure 2*a*. This series is not deeply stained, so that it is somewhat difficult to make out distinct differences in the density of the mesenchyme. The anlagen of the vertebra, therefore, not very distinct. The undulations of the notochord described by Minot well shown. The denser mesenchyme of the anlagen of the vertebra extends forward into the denser mesenchyme found ventral to the brain tube, the anlage of the basal plate. The notochord just after it bends ventrally into the retropharyngeal region comes in contact with the pharyngeal epithelium which is distinctly thickened in the area of contact, (fig. 2*a*) upper right hand portion of figure. For a distance cephalad of this area of contact it is somewhat difficult to make out clearly the course of the notochord and its relation to the pharyngeal epithelium. It appears to course just over the epithelium, with here and there a mesenchymal cell between. The want of distinctness of the notochord in this region is partly due to the fact that the pharyngeal epithelium presents a distinct kink dorsalward, situated a short distance cephalad of the thyroglossal pit in the tongue, in which region all of the retropharyngeal structure are bent dorsally. The arrangement and apparent compression of the mesenchyme suggests that this kink is an artefact. Just cephalad of this kink the notochord is again clearly made out, and here again touches the pharyngeal epithelium just before it bends dorsally to end in the denser mesenchyme of the basal plate anlage. Just before the cephalic end of the notochord is reached it presents a ventral off-shoot, which is, however, not clearly followed to its termination. This off-shoot seems to me similar to the ones described by Grünwald for sheep embryos as the remains of the ventral extensions of the notochord which ends in Seessel's pocket.

Embryo C, No. 389, Mall collection, 8 mm. Figure 3 and figure 3*a*. This embryo is slightly macerated, as indicated by the Retzius folds in the brain tube and the appearance of the mesenchyme. The pharyngeal epithelium not macerated and in place. The vertebral anlagen

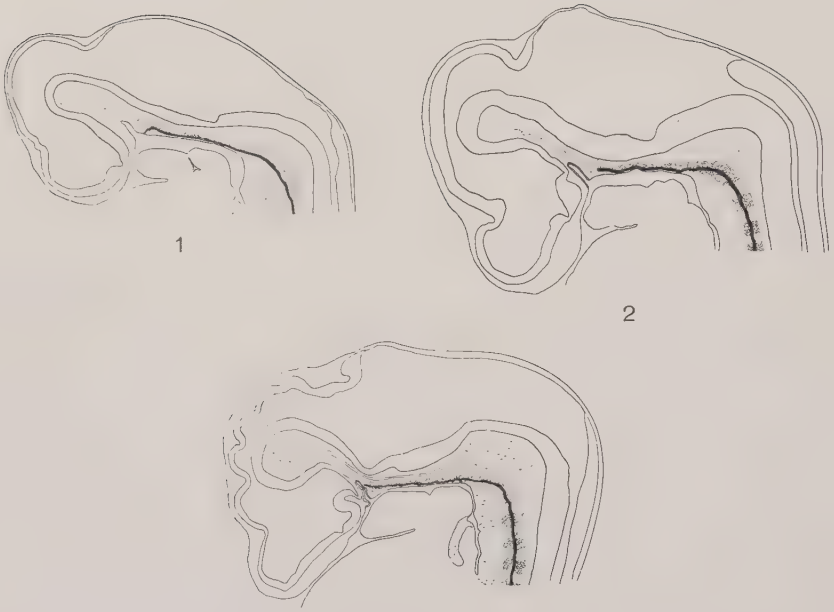


Fig. 1 No. 371, Mall collection, Embryo *A*, 6.6 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 10.

Fig. 2 No. 221, Mall collection, Embryo *B*, 7.5 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 10.

Fig. 3 No. 389, Mall collection, Embryo *C*, 8 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 10.

appear as distinct blocks of denser mesenchyme which extends forward as a denser layer of mesenchyme, the anlage of the basal plate. The notochord makes a distinct loop just after it bends ventrally to pass into the retropharyngeal region beneath the anlage of the basal plate. The end of this loop is bifid, both parts coming in contact with the pharyngeal epithelium. The notochord then courses along over the pharyngeal epithelium, presenting slight undulations, and here and there just touching the pharyngeal epithelium, then turns dorsally with rather irregular contour, to end near the top of Rathkes pouch, as shown in figure 3. All along its course in the retropharyngeal region mesenchyme is found between the chorda and the pharyngeal epithelium. The relation of the notochord, as also the distinct loop and region of contact between chorda and the pharyngeal epithelium is clearly shown in figure 3*a*.

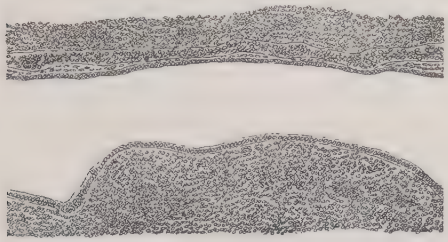


Fig. 1*a* No. 371, Mall collection, Embryo *A*, 6.6 mm. Drawing shows vault of pharynx and dorsum of tongue. Notochord in contact with pharyngeal epithelium near upper right portion of figure. × 100.

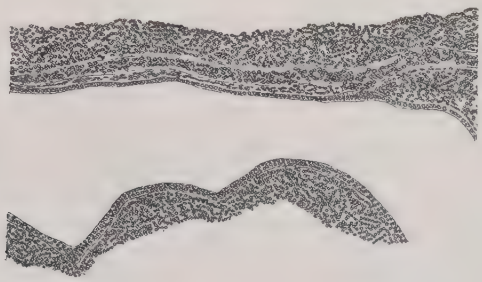


Fig. 2*a* No. 221, Mall collection, Embryo *B*, 7.5 mm. Drawing shows vault of pharynx and dorsum of tongue. Notochord in distinct contact with pharyngeal epithelium, which is thickened in area of contact. × 100.

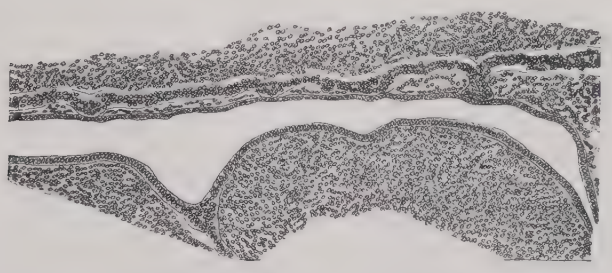


Fig. 3*a* No. 389, Mall collection, Embryo *C*, 8 mm. Drawing shows vault of pharynx and dorsum of tongue. Notochord makes distinct loop—upper right hand portion of figure—and comes in contact with the pharyngeal epithelium. Other less distinct points of contact of chorda and the pharyngeal epithelium shown. × 100.

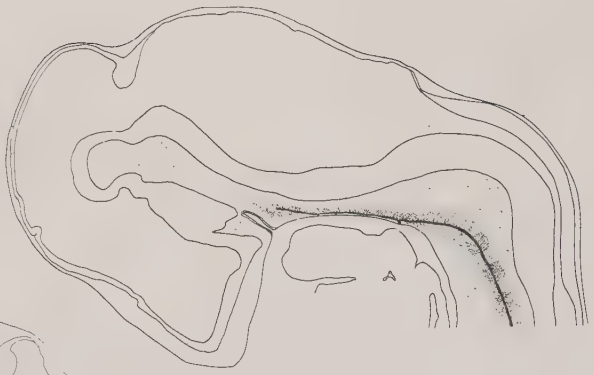
Embryo D, No. 3, Huber collection, 10 mm. Figure 4 and figure 4*a*. Very well preserved human embryo, cut in sagittal sections of 5μ thickness. Vertebral anlagen present as very distinct blocks of denser mesenchyme. Denser mesenchyme extends cephalad as the anlage of the basal plate. Pharyngeal epithelium well preserved. The notochord as it bends ventrally to pass into the retropharyngeal region, beneath the anlage of the basal plate forms a distinct loop which comes in contact with the pharyngeal epithelium. In this area the epithelium presents two irregular rows of nuclei, while for the remainder of the pharyngeal vault the epithelium is simple, with a single row of nuclei. In the series of sections the caudal portion of the looped notochord appears first. The cephalic end of the loop begins to appear in the fourth following section, showing that the chorda at the bottom of the loop is also bent laterally. Figure 4*a* represents a reconstruction drawing made from the three most favorable sections of this region. This drawing does not show as clearly, as may be seen by tracing through the series the direct contact between chorda and pharyngeal epithelium, which contact is most distinct against the side of a small fold of pharyngeal epithelium, difficult to represent in the drawing. Cephalad to this region, owing to a slight deviation of the head from the mid-plane, the notochord is traced, obliquely cut, through 25 sections of the series to its end near the upper end of Rathkes pouch. Near its anterior end the notochord, just before it turns dorsally again touches the pharyngeal epithelium in two places, for the remainder of its course in the retropharyngeal region it is distinctly separated from the epithelium by a small amount of mesenchyme.

Embryo E, Mall collection, No. 406, 13 mm. Figure 5 and figure 5*a*. Well stained and well preserved series. Practically the whole of the head chorda, from where it leaves the anlagen of the vertebra to its cephalic end embraced in one section. Vertebral anlagen and basal plate in stage of precartilage, and clearly outlined. As the notochord bends ventrally to pass into the retropharyngeal region beneath the anlage of the basal plate it presents a trilobed ventral extension, which extends practically to the pharyngeal epithelium, in a region just over the thyroglossal pit in the tongue. A few mesenchymal cells have grown in between this ventral extension of the chorda and the pharyngeal epithelium, so that it seems even in this region completely separated from the pharyngeal epithelium, though in very close relation with it (fig. 5*a*). In the remainder of its course in the retropharyngeal region the notochord follows closely along the ventral side of the denser mesenchyme bordering the precartilage of the basal plate, and is separated

by several layers of mesenchymal nuclei from the pharyngeal epithelium. Near its cephalic end the notochord bends dorsally to reënter the anlage of the basal plate, in which it terminates near Rathkes pouch. The pharyngeal vault of this embryo presents a distinct dorsal kink, somewhat cephalad of the thyroglossal pit of the tongue. The notochord and mesenchyme are likewise bent dorsally in this region, as may be seen from figure 5. This is looked upon as an artefact.

Embryo F, Huber collection, No. 4, 15 mm. Figure 6 and figure 6*a*. Vertebral anlagen in stage of embryonic cartilage, as also basioccipital. The notochord as it leaves the vertebral anlagen passes dorsal to the cartilaginous basioccipital, through the caudal end of which it bends ventrally to reach the retropharyngeal region. Just after it reaches this region the notochord presents a distinct, triangular shaped, ventral extension, which comes in contact with a shallow pit lined by a stratified pharyngeal epithelium, a pit which is regarded as the anlage of the pharyngeal bursa. This ventral chordal extension does not rest on the top of the infolded pharyngeal pit, but a little to its side, so that the chordal extension and the pharyngeal pit appear in the four sections preceding the one from which figure 6*a* was drawn. In two of these sections the contact between chordal and pharyngeal epithelium is intimate, much more so than in the section from which the figure was taken. The condition here presented appears to me very similar to that found in Embryo *I*, of Linck's series, except that the pharyngeal epithelium in Embryo *F*, is in excellent state of preservation, so that its relation to the chordal cells could be clearly made out. In this preparation there is no question of the direct contact of the notochord with the pharyngeal epithelium in the region of the forming pharyngeal bursa. A slight distance cephalad of this region the notochord again comes in contact with the pharyngeal epithelium, presenting two ventral extensions with common base, both extensions reaching the epithelium which in this region is also thickened, however, there is no evagination. The notochord then passes along the ventral surface of the basioccipital, presenting three further ventral extensions which do not reach the pharyngeal epithelium, then bends dorsally to reënter the basioccipital in which it ends near the hypophysial anlage, which shows the first stages of separation from the oral epithelium, in that the dorsal vesicular portion is attached to the oral epithelium by means of a solid chord of cells.

Embryo G, Huber collection, No. 6, 16 mm. Figure 7 and figure 7*a*. The vertebral anlagen and basioccipital in stage of embryonic cartilage, the dens epistrophei outlined in dense mesenchyme, the hypochordal arch in precartilage. The head chorda almost through its entire length



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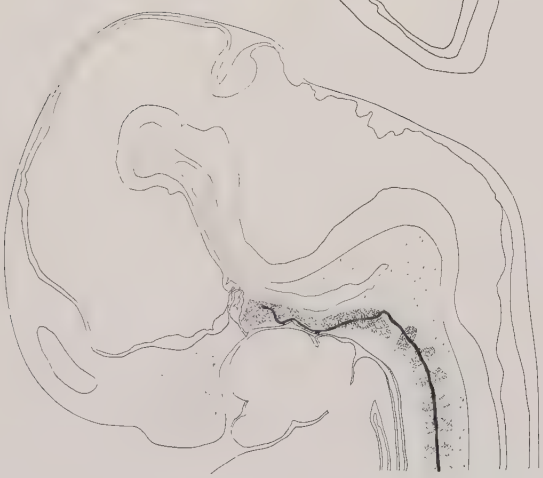

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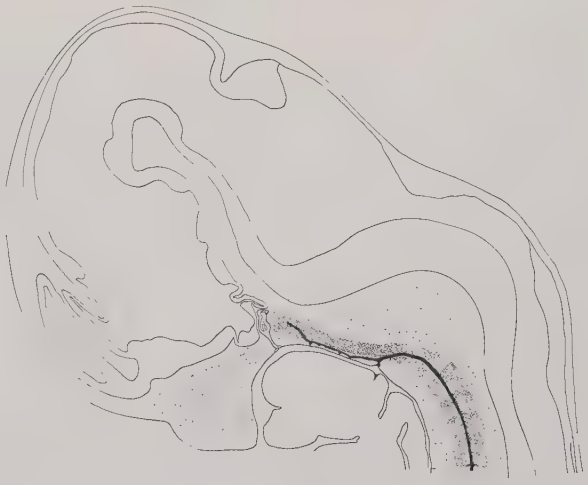

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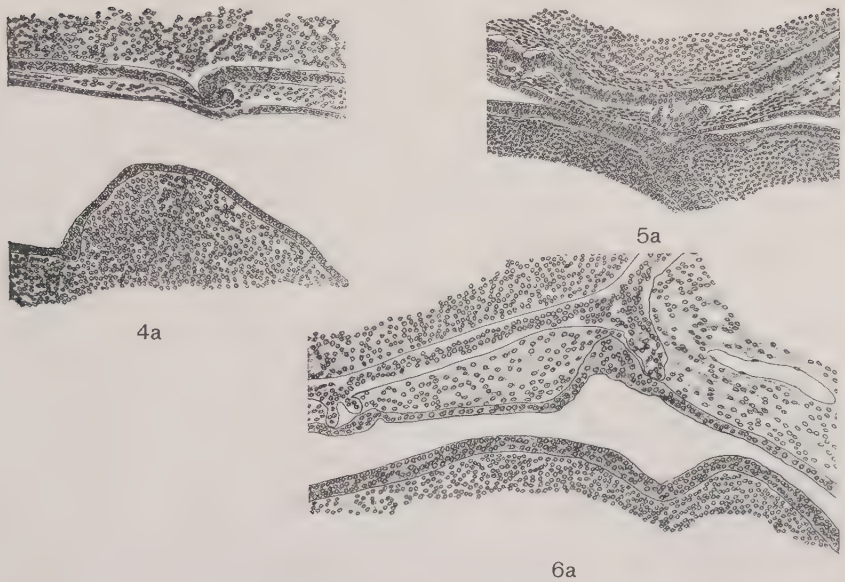


Fig. 4 No. 3, Huber collection, Embryo *D*, 10 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 10.

Fig. 4*a* No. 3, Huber collection, Embryo *D*, 10 mm. Drawing of portion of vault of pharynx and dorsum of tongue. Notochord shows distinct loop in contact with pharyngeal epithelium. × 100.

Fig. 5 No. 406, Mall collection, Embryo *E*, 13 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 10.

Fig. 5*a* No. 406, Mall collection, Embryo *E*, 13 mm. Drawing shows portion of vault of pharynx and dorsum of tongue. Distinct ventral extension of notochord, just over thyroglossal pit in tongue, which is not quite in contact with pharyngeal epithelium, a few mesenchymal cells intervening. × 100.

Fig. 6 No. 4, Huber collection, Embryo *F*, 15 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 7.5.

Fig. 6*a* No. 4, Huber collection, Embryo *F*, 15 mm. Drawing showing portion of vault of pharynx and dorsum of tongue. Distinct ventral extension of notochord in contact with an evagination of the pharyngeal epithelium the anlage of a pharyngeal bursa. Further point of contact of notochord and pharyngeal epithelium to the left in figure. × 100.

is embraced in one section. The notochord surrounded by a distinct chordal sheath passes obliquely through the caudal portion of the basi-occipital to reach the retropharyngeal region in which region, through its entire course, it is in close relation with the ventral surface of the basi-occipital. Just over the region of the thyroglossal pit in the tongue, the notochord presents a bilobed, ventral extension, which passes obliquely

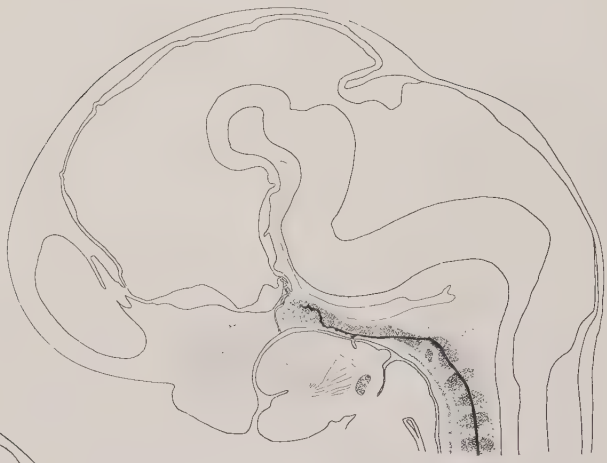
in a cephalad direction to near the pharyngeal epithelium from which it is separated, however, by a layer of mesenchymal cells. The pharyngeal epithelium of this region is not evaginated, nor is it thickened; there is no evidence, therefore, of the anlage of a pharyngeal bursa. The notochord presents several ventral bulgings which do not reach the pharyngeal epithelium before it bends dorsally to end in the basioccipital near the anlage of the hypophysis, which is almost completely separated from the oral epithelium.

Embryo H, Huber collection, No. 32, 19 mm. Figure 8 and figure 8*a*. This embryo was slightly macerated, as is shown by the Retzius folds of the brain vesicles, not reproduced in figure 8. Pharyngeal epithelium and mesenchymal structures fairly well preserved, epithelium in place. The vertebral anlagen and basioccipital in stage of embryonic cartilage and well outlined. The notochord after leaving the vertebral anlagen passes obliquely through the caudal end of the basioccipital to reach the retropharyngeal region. Almost immediately after reaching the ventral surface of the basioccipital the notochord presents two ventral extensions. The posterior of these is of leaf-shape and does not quite reach the pharyngeal epithelium, while the anterior of the two extensions is of cylindrical shape and comes in contact with the epithelium of an evaginated portion of the pharyngeal wall, the anlage of a pharyngeal bursa. The middle of the bursa is not cut in the section figured, so that the epithelium appears greatly thickened, due to the fact that it is in part cut tangentially. It is for this reason also difficult to speak with certainty of the exact relations presented by the chordal epithelium. It would appear, however, that the chordal epithelium is in direct contact with the pharyngeal epithelium, without the intervention of mesenchyme. The notochord presents another, a club-shaped ventral extension slightly cephalad to the pharyngeal bursa, which does not reach to pharyngeal epithelium, being separated from it by a layer of mesenchyme. Then after coursing along the ventral surface of the basioccipital for a short distance the notochord bends dorsally to end in this in the region of the hypophysial anlage. The craniopharyngeal canal is nearly obliterated, containing a slender interrupted chord of hypophysial cells. Above the seat of the original attachment of the oral hypophysis anlage which is still evident in the oral epithelium there is found in the mesenchymal mucosa a small mass of hypophysial epithelium, a remnant of the hypophysial duct.

Embryo I, Huber collection, No. 33, 25 mm. Figure 9 and figure 9*a*. Embryo slightly macerated, as is shown by the Retzius folds in the

brain vesicles, not shown in figure 9. Epithelium slightly macerated, though in place over the greater part of the pharyngeal vault. Vertebral anlagen and the basioccipital in cartilage stage, dens epistrophei and hypochordal arch in stage of embryonic cartilage The notochord with well developed chordal sheath can be traced as a continuous chord somewhat retracted from the sheath, from the dens epistrophei to the dorsal surface, thence to the ventral surface of the basioccipital, through the caudal end of which it passes obliquely, to extend along its ventral surface for a short distance. From this region there extends ventrally a slender extension surrounded by a thin chordal sheath, which may be traced nearly to the blind end of a pharyngeal evagination, with narrow lumen, and of cylindrical shape, the anlage of a pharyngeal bursa. The chordal cells cannot be traced quite to the pharyngeal epithelium, though no mesenchyme intervenes. The impression is left that during fixation the two parts had separated, owing to contraction. Cephalad to the region of the pharyngeal bursa the notochord was fixed in processes of breaking down, though if many sections are combined a continuous structure may be made out, as is shown in figure 9. The section from which figure 9*a* was taken, that section in which the pharyngeal bursa was most clearly shown, presents the notochord cut twice in the retropharyngeal tissue, the thick chordal sheath may here be seen, as also the contracted chordal tissue. The notochord finally bends dorsally, to end in the basioccipital some little distance from the hypophysis. The craniopharyngeal canal is practically obliterated. The place of origin of the hypophysis is marked in the oral epithelium by a slight evagination. Just dorsal to this end and still attached to the oral epithelium there is found in the mucosa, a vesicle of oval shape, epithelium lined, developed from the hypophysial stalk.

Embryo J, Huber collection, No. 47, 32 mm. Figure 10 and figure 10*a*. This embryo which was received while still warm, from the service of Professor Peterson (hysterectomy for large fibroma), in excellent state of preservation, was cut into a practically faultless series of sagittal sections of 15μ thickness. The cartilaginous cranium well developed. The notochord after leaving the dens epistrophei takes a tortuous course to the dorsal surface of the caudal end of the basioccipital. In its course through the cartilage the notochord may be traced by an empty though well developed chordal sheath. The epithelium of the chorda appears again as the ventral surface of the basioccipital is reached, and may in the retropharyngeal tissue be traced to the blind end of a deep, cylindrical shaped evagination of the pharyngeal epithelium, a well developed pha-



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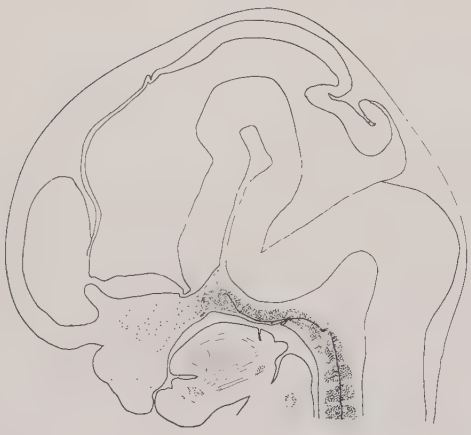

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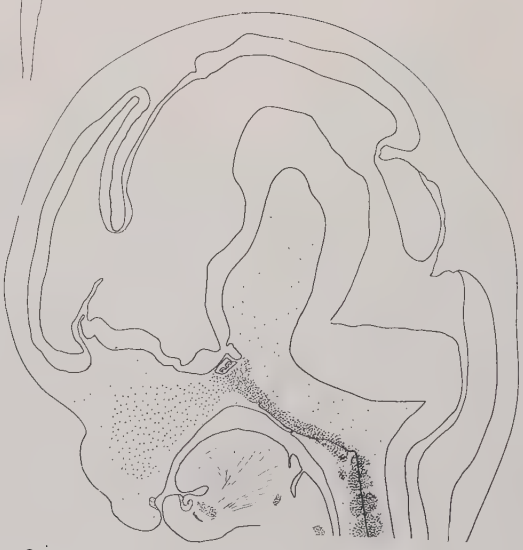

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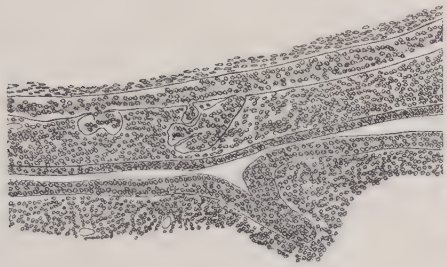

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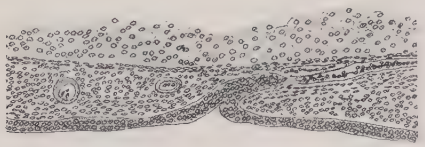

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Fig. 7 No. 6, Huber collection, Embryo *G*, 16 mm. Reconstructed outline drawing of midplace sagittal section of head. Notochord black. × 7.5.

Fig. 7*a* No. 6, Huber collection, Embryo *G*, 16 mm. Drawing shows portion of vault of pharynx and dorsum of the tongue. Distinct, bilobed ventral extension of the notochord, just dorsal to the thyroglossal pit in tongue, which does not quite reach the pharyngeal epithelium, which is neither distinctly thickened nor pitted in this region. × 100.

Fig. 8 No. 32, Huber collection, Embryo *H*, 19 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 7.

Fig. 8*a* No. 32, Huber collection, Embryo *H*, 19 mm. Drawing shows a portion of vault of pharynx, the anlage of a pharyngeal bursa, and two ventral extensions of the notochord the more slender of which is in contact with the pharyngeal epithelium of the bursa. × 100.

Fig. 9 No. 33, Huber collection, Embryo *I*, 25 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 7.

Fig. 9*a* No. 33, Huber collection, Embryo *I*, 25 mm. Drawing shows a portion of the pharyngeal vault including the ventral part of the basioccipital in stage of embryonic cartilage. A distinct, slender pharyngeal bursa. The notochord, ventral to the cartilage—right end of figure—comes in contact with blind end of bursa. Notochord cut twice to the left of bursa, as seen in figure. × 100.

ryngeal bursa, well shown in figure 10*a*, drawn from the section in which the pharyngeal bursa was most clearly portrayed. In its course through this portion of the retropharyngeal region the notochord is closely surrounded by dense strands of developing connective tissue which run parallel with it. It is not possible to trace the chordal epithelium to a contact with the pharyngeal epithelium, although the chordal sheath may be

traced to the immediate vicinity of the pharyngeal epithelium, so that it becomes attached to the blind end of the pharyngeal bursa by means of the connective tissue strands above referred to. This may be more clearly seen in the two sections preceding the one from which figure 10*a*, was drawn, in which only a portion of the chordal sheath surrounded by parallel strands of connective tissue may be seen, upper portion of figure. In the retropharyngeal region cephalad to the pharyngeal bursa the notochord is present in the form of three relatively large clumps of chordal tissue, the most anterior of which, just before the chorda again bends dorsally, touches the pharyngeal epithelium which is here also slightly evaginated. The chorda ends in the basioccipital near the hypophysis anlage, and in its course from the retropharyngeal region to its termination, consists of a continuous chord of chordal cells sursounded by a well developed chordal sheath. The craniopharyngeal canal is practically obliterated, although the place of origin of the hypophysis from the oral epithelium is still evident, and in the mucosa just above this a relatively large mass of hypophysial tissue is found, still attached to the oral epithelium.

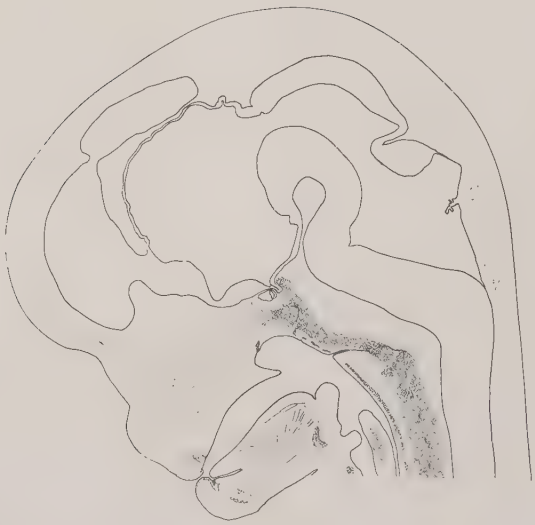
Embryo K, Huber collection, No. 49, 47 mm. Well preserved embryo. Basioccipital shows early stages of bone development. Nearly the entire head chorda has disappeared. The original course through the caudal end of the basioccipital can be traced by a band of homogeneous matrix free from cartilage cells. No trace of notochord in the retropharyngeal region. There is present a shallow, but well developed pharyngeal bursa, to which may be traced strands of dense fibrous tissue, coming from that region of the basioccipital which contained the place of exit of the notochord in its course into the retropharyngeal region. A portion of the cephalic end of the notochord, having the form of a *Y*, is found in the basioccipital near the region of the hypophysis. The larger arm of this *Y*-shaped structure points ventrally toward the retropharyngeal region, one of the shorter arms toward the hypophysis, the other reaches the dorsal perichondrium a little caudal to the sella turcica.

Embryo L, Huber collection, No. 23, 60 mm. Figure 11 and figure 11*a*. The sagittal series embraces that portion of the embryo extending from the base of the skull to the diaphragm, cut in sections of 15μ thickness. Tissue well preserved. Early stages of bone development in the caudal end of the basioccipital. A large irregularly shaped mass of chordal tissue is found just cephalad of the dens epistrophei and dorsal to the caudal end of the basioccipital. The course of the chordal canal through the caudal end of the occipital cartilage is evident, but no chordal

tissue is met with until the ventral surface of the cartilage is reached. There is a small mass of chordal epithelium embedded in the perichondrium. Dorsal and slightly caudal to a well developed pharyngeal bursa there is observed a large, irregularly shaped mass of chordal tissue, which though not in contact with the epithelium of the pharyngeal bursa, is attached to the blind end of the bursa by means of strands of fibrous connective tissue, as shown in figure 11*a*. This chordal mass presents a slender ventrally projecting portion which in other sections of the series may be traced to nearly the pharyngeal bursa. The retropharyngeal region contains three other relatively large masses of chordal tissue, which, however, do not come in contact with the pharyngeal epithelium. The cephalic end of the notochord ends in the basioccipital some little distance from the sella turcica. The general relations of the chordal remains in the retropharyngeal region are shown in figure 11.

Embryo M, Huber collection No. 27, 100 mm. Figure 12. That portion of this embryo extending from the base of the skull to the diaphragm cut in sagittal sections of 15μ thickness. Tissue well preserved. Caudal end of basioccipital in stage of endochondral bone development, center of ossification of the body of the sphenoid indicated. Practically the entire head chorda has disappeared. There is observed a relatively large mass of chordal tissue lying dorsal to the caudal end of the basioccipital, as shown in figure 12. The retropharyngeal region is free from chordal remains with the exception of a small mass lying against the perichondrium in the region where in earlier stages the chorda turned dorsally to reënter the basioccipital, just dorsal to this, several detached masses of chordal tissue are found in the cartilage some little distance from the sella turcica. There is found a very shallow depression of the pharyngeal wall which from its position and its relation to the upper border of the pharyngeal constrictor muscles must be looked upon as an imperfectly developed pharyngeal bursa. Toward this slight depression there extends a band of denser connective tissue which has its origin in the periosteum near the caudal end of the basioccipital, a band which Froriep has designated as the Lig. occipito-pharyngeum. In the mucosa ventral to the cartilage beneath the sella turcica there is found a relatively large mass of hypophysial tissue of cylindrical shape, and still attached to the lining epithelium.

Embryo N, Huber collection, No. 29, 135 mm. Figure 13. That portion of this embryo extending from the base of the skull to the thyroid region in the neck cut in sagittal sections of 20μ thickness; slightly macerated, the epithelium in part attached. The greater part of the basi-



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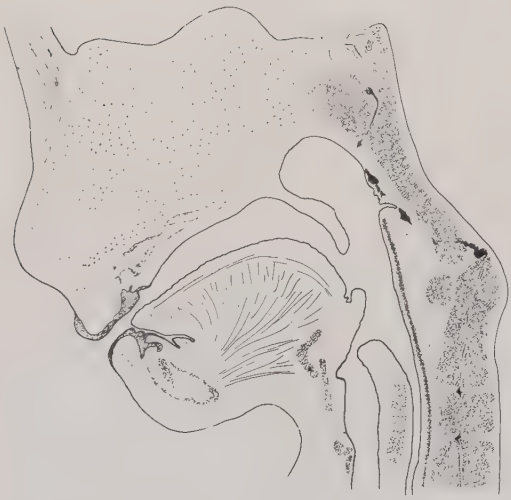

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Fig. 10 No. 47, Huber collection, Embryo *J*, 32 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochordal remains black. Distinct pharyngeal bursa just cephalad to the upper border of the pharyngeal constrictors. × 5.

Fig. 11 No. 23, Huber collection, Embryo *L*, 60 mm. Reconstructed outline drawing of midplane sagittal section of portion of head. Notochordal remains black. Deep pharyngeal bursa situated just cephalad of the upper border of pharyngeal constrictors. × 5.

Fig. 10*a* No. 47, Huber collection, Embryo *J*, 32 mm. Drawing shows the deep pharyngeal bursa and surrounding retropharyngeal mucosa. Upper portion of figure shows a thickened chordal sheath accompanied by parallel strands of fibrous tissue, reaching the blind end of the bursa. × 100.

Fig. 11*a* No. 23, Huber collection, Embryo *L*, 60 mm. Drawing shows vault of pharynx in region of the pharyngeal bursa. Large mass of notochordal tissue dorsal to bursa and united to bursa by strands of fibrous tissue having a parallel course. Smaller mass of chordal tissue, lying near pharyngeal epithelium, to the left in figure. × 100.

occipital shows endochondral and periosteal bone development; the body of the sphenoid shows endochondral bone. All traces of the head chorda have disappeared with the exception perhaps of a remnant situated in a relatively large space in the cartilage, slightly caudal to the sella turcica. This embryo presents a well developed pharyngeal bursa situated just cephalad to the upper border of the pharyngeal constrictor muscles. Its relations are shown in figure 13. The epithelium lining the

bursa is stratified and presents especially on its posterior wall patches of stratified ciliated columnar epithelium interspersed in the stratified pavement epithelium. The connective tissue surrounding the bursa contains many nuclei, not arranged, however, to form an adenoid tissue.

Embryo O, Huber collection, No. 30, 145 mm. Figure 14 and figure 14*a*. That portion of the embryo extending from the base of the skull to the thyroid region in the neck cut in sagittal sections of 15μ in thickness; slightly macerated, epithelium in part detached. Ossification about as in Embryo *N*, except that it is further advanced in the body of the sphenoid. No traces of head chorda present. The mucosa ventral to the sella turcica contains a relatively large mass of hypophysial tissue which is arranged in epithelial cords and closed vesicles. There is present a well developed pharyngeal bursa situated just cephalad of the upper border of the pharyngeal constrictor muscles, as is shown in figure 14. The epithelium lining the bursa is stratified, its posterior wall showing patches of stratified ciliated columnar epithelium interspersed between areas of stratified pavement epithelium. The blind end of the pharyngeal bursa is slightly invaginated by a well defined mass of adenoid tissue, the anlage of the pharyngeal tonsil. In figure 14*a* is shown the upper end of the bursa with the contiguous adenoid tissue. Attention should be called to the fact that the posterior wall of the bursa is directed to the left in figure 14*a*, the reverse of that shown in figure 14.

By way of comparison and to emphasize the fact that it is necessary to have at one's disposal human embryos in order to study in detail special structures in their development in man, I present here reconstruction drawings of the heads of rabbit, pig and rat embryos of a stage in which the vertebral anlagen and the basioccipital present the structures of embryonic cartilage, and in which the head chorda may be traced throughout its entire extent.

Rabbit embryo, Series L. Emb. coll. Depart. Hist. and Emb., U. of M., 17 mm. crown-rump length, figure 15. Vertebral anlagen and basioccipital in the stage of embryonic cartilage. Oral portion of hypophysis still attached to the chordal epithelium by a cord of cells. Slightly caudal to this oral attachment of the hypophysis a distinct evagination of epithelium, which for the present I may regard as the remains of Seessel's pocket. The notochord passes in a tortuous course from the tip of the dens epistrophei to the dorsal surface of the caudal end of the basioccipital, then passes obliquely through this to reach the ventral surface of the

cartilage between which and the dense perichondrium it courses to near its cephalic end. It then turns dorsally, reëntering the basioccipital cartilage, to terminate in contact with the perichondrium lining the posterior wall of the sella turcica. Nowhere does the notochord reach the retropharyngeal tissue ventral to the perichondrium of the basioccipital.

Pig embryo, No. 9, Emb. coll. Depart. Hist. and Emb. U. of M., 20 mm. crown-rump length, figure 16. Vertebral anlage and basioccipital in stage of embryonic cartilage. Hypophysis just after complete separation from oral epithelium. The notochord after passing through dens epistrophei passes to the dorsum of the caudal end of the basioccipital, into which it passes obliquely, descending to about the middle of the cartilage and presenting a tortuous course. About midway between the caudal and the cephalic end of the basioccipital the notochord approaches the ventral side of the cartilage, however, is surrounded on all sides by cartilage. From here the notochord bends dorsally in a long curve, to terminate under the perichondrium of the posterior wall of the sella turcica. In the pig embryo of 30 mm. head-rump length used by Mead in his study of the chondrocranium, the notochord reached the retropharyngeal region and was interrupted by infoldings of the pharyngeal epithelium. Mrs. Gage states that in about 20 per cent of pig embryos studied by her, the notochord came in contact with a mouth pocket similar to that found in man. None of the embryos of my series of pertinent stages, though the series is not extensive, show this, but present about the relations of the head chorda shown in figure 16.

Rat embryo, No. 9, Emb. coll. Depart. Hist. and Emb. U. of M., 9 mm. crown-rump length, figure 17. Vertebral anlagen and basioccipital in stage of embryonic cartilage, though the cells are beginning to be separated by matrix. Hypophysis just after complete separation from oral epithelium. Just caudal to the place of attachment of the oral portion of the hypophysis, still evident in the series, there is observed a distinct evagination of the lining epithelium, which for the present may be regarded as the remains of Seessel's pocket. The notochord after leaving the dens epistrophei passes to the dorsal surface of the caudal end of the basioccipital, and then extends cephalad along its dorsal surface just beneath the perichondrium, and in part embedded in the perichondrium, to near the caudal border of the sella turcica. At no place is the notochord embedded in the cartilage of the basioccipital. This is the usual course of the head chorda in the series of rat embryos at my disposal. It is evident, therefore, that in the three forms here

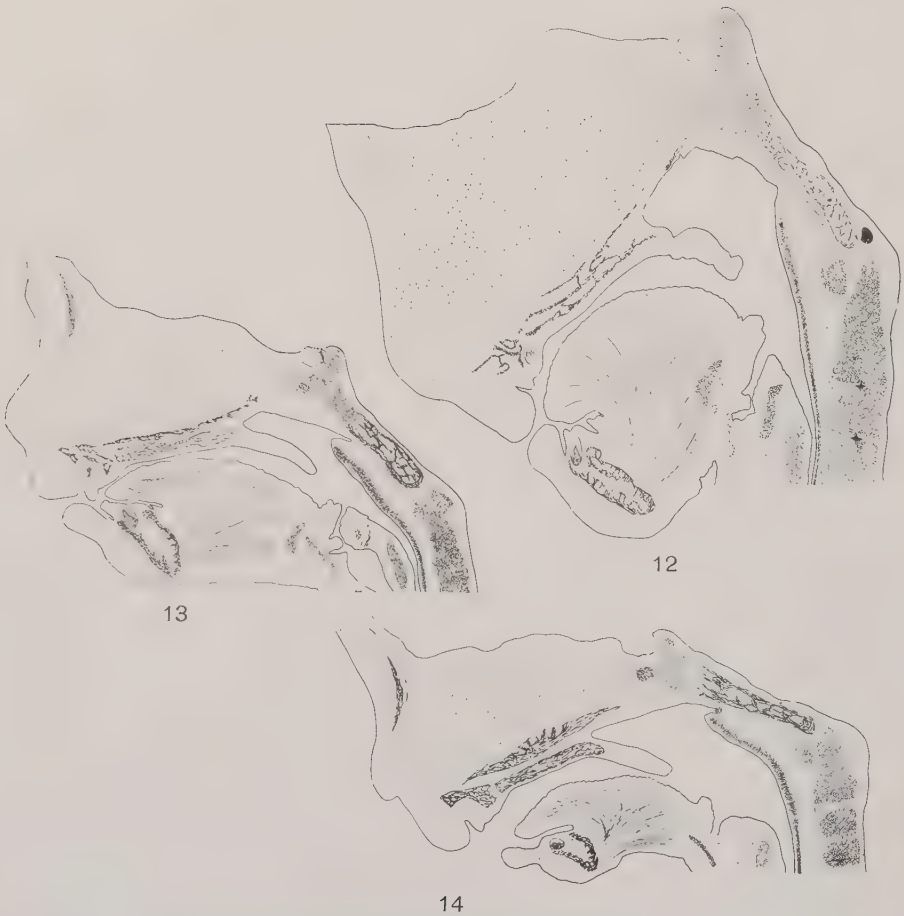
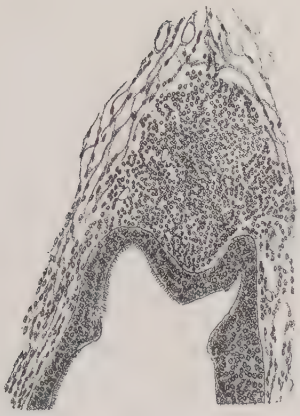


Fig. 12 No. 28, Huber collection, Embryo *M*, 100 mm. Reconstructed outline drawing of midplane sagittal section of portion of head. Notochordal remains black, dorsal to caudal end of the basioccipital. Shallow pharyngeal bursa just cephalad of upper border of pharyngeal constrictors. Distinct Lig. occipitopharyngeum of Froriep. × 4.

Fig. 13 No. 29, Huber collection, Embryo *N*, 135 mm. Reconstructed outline drawing of midplane sagittal section of portion of head. Distinct pharyngeal bursa situated just cephalad of the upper border of the pharyngeal constrictors. × 2.

Fig. 14 No. 30, Huber collection, Embryo *O*, 145 mm. Reconstructed outline drawing of midplane sagittal section of portion of head. Distinct pharyngeal bursa, with anlage of the pharyngeal tonsil, situated just cephalad of the upper border of the pharyngeal constrictors. × 2.



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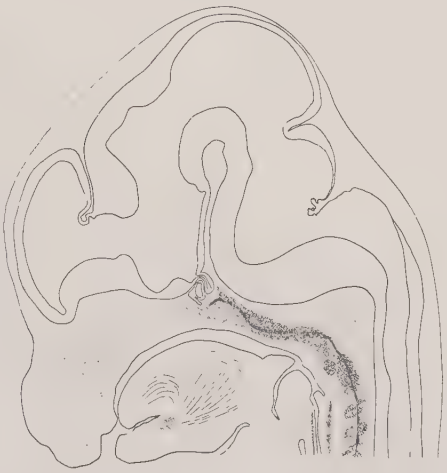

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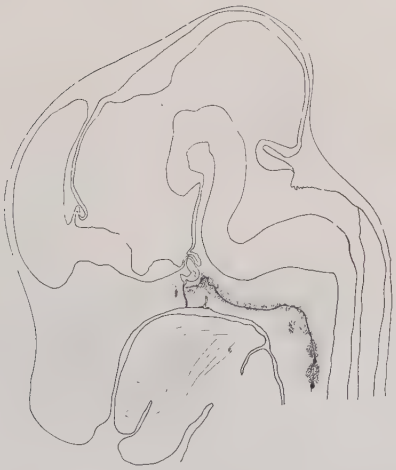

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Fig. 14*a* No. 30, Huber collection, Embryo *O*, 145 mm. Drawing shows the dorsal part of the pharyngeal bursa with the anlage of the pharyngeal tonsil. × 100.

Fig. 15 Rabbit embryo, crown-rump length 17 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black, hypophysis still attached to epithelium. × 7.5.

Fig. 16 Pig embryo, crown-rump length 20 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 7,5.

Fig. 17 Rat embryo, crown-rump length 9 mm. Reconstructed outline drawing of midplane sagittal section of head. Notochord black. × 7.5.

presented, with possible exception in pig embryos, the head chorda has a course which is very different from that noted in human embryos, and furthermore that its course is characteristic in each of the forms presented; in rabbit embryos, along the ventral surface of the cartilage of the basioccipital, in pig embryos, again with possible exceptions, through about the middle of the cartilage of the basioccipital, and in rat embryos along the dorsal surface of the basioccipital. Any attempt to draw deductions as to the relations of the head chorda in man based on observations made on the mammalian embryos noted, therefore, would be futile and misleading.

The material presented, it seems to me, shows conclusively that there exists in human embryos a distinct relation between the head notochord and the pharyngeal entoderm in the development of the pharyngeal bursa, as was first demonstrated by Froriep, confirmed by Nebelthau, Mrs. Gage, Meyer, Linck. In the earlier stages of development of the human embryo, in embryos having head breech length of from 5 mm. to 8 mm., a time when the head chorda is separating distinctly from the pharyngeal entoderm, one may determine a small area in which the notochord remains in close contact with the pharyngeal entoderm, in which area the pharyngeal epithelium shows a distinct reaction evinced by an increased thickness of its cells. This area is situated, in the stages under discussion, some distance caudal to the region of the thyroglossal pit in the tongue, although the distance between the points varies somewhat in different embryos. This area of contact of chordal and pharyngeal epithelium is found at the end of the ventral flexure of the notochord as this bends ventrally to pass from the vertebral anlagen to the retropharyngeal region. If several points of contact are noted, as is often the case in these earlier stages, the special area above mentioned is the most caudal and is constant both as to character and position, and represents, I am convinced, the seat of the development of the pharyngeal bursa. This area is separated from the region of Rathke's pouch and Seessel's pocket by practically the whole length of the future pharyngeal vault, as is clearly shown in Embryo *A*, of this series (fig. 1). The contention of Meyer that the pharyngeal bursa develops from the region of Seessel's pocket is not confirmed, there-

fore, by my own observations. In human embryos having a crown-breech length of from 8 mm. to 15 mm., spanning a period in which the dense mesenchyme of the ventral anlagen and the basioccipital develops to the stage of precartilage or embryonic cartilage, and in which the notochord becomes distinctly separated from the pharyngeal epithelium in the retropharyngeal region by ingrowth of mesenchyme, the notochord presents a distinct loop just cephalad of the ventral flexure by means of which it passes from the vertebral anlagen to the retropharyngeal region ventral to the basioccipital. This loop of the chorda, always distinct, is situated a little caudal to the region of the thyroglossal pit in the tongue, for the earlier stages, and just about dorsal to this pit for the older stages of the human embryos varying in length of from 8 mm. to 15 mm. showing that the area of distinct contact of chordal and pharyngeal epithelium, the area where the pharyngeal bursa develops, changes it relative position as the embryo proceeds in development. In one of the embryos of my series, Embryo *F*, this ventral loop of the notochord has already separated from the pharyngeal epithelium indicating a looser connection between chordal and pharyngeal epithelium than in other embryos in which this connection persists into older stages. All observers who have studied the development of the embryonic pharyngeal bursa state that this is not present in all embryos. The percentage of embryos in which the embryonic pharyngeal bursa is present or absent, as given by these different observers, varies. The data given are not comparable, since embryos of different ages or lengths are used by the several observers dealing with this question. Suffice it to say that all observers who have especially studied the development of the pharyngeal bursa have noted its absence in a certain percentage of embryos studied. It is, therefore, of interest to note that the connection between notochord and pharyngeal epithelium may be so loose in certain embryos that a complete separation is attained in early stages, as in Embryo *F*, 13 mm., the ingrowth of the mesenchyme into the retropharyngeal region being sufficient to completely separate the chorda from the pharyngeal epithelium. In my series of human embryos having a length varying from 15 mm, to about

30 mm there was present a distinct embryonic pharyngeal bursa in all except Embryo *G*, and in all except this embryo did the ventral extension of the notochord come in close relation or in direct contact with the epithelium of the blind end of the pharyngeal bursa. In Embryo *G*, there is present a distinct bilobed ventral extension of the notochord, situated just dorsal to the thyroglossal pit of the tongue, the region of contact of the chordal and pharyngeal epithelium in case a pharyngeal bursa develops, which, however, was lacking in this embryo, neither was the pharyngeal epithelium of the region thickened. This embryo I interpret as again showing an early separation of head chorda and pharyngeal epithelium, owing to a loose connection between the two structures. Of the older stages presented all but Embryo *M* show a distinct pharyngeal bursa. In this embryo in which there was present a shallow depression, which may be regarded as an imperfectly developed pharyngeal bursa, in that it is situated just cephalad to the upper border of the pharyngeal constrictors, a relation characteristic for older stages, there is present a distinct occipito-pharyngeal ligament, which ligament was regarded by Froriep as a possible caustive factor in the development of the pharyngeal bursa. The fact that in this embryo there is present only an imperfectly developed pharyngeal bursa on the one hand and on the other hand an especially well developed occipito-pharyngeal ligament, would seem to argue against regarding this ligament as a causative factor in the development of the pharyngeal bursa. In the older stages presented, the head chorda is in process of disintegration, or has entirely disappeared from the retropharyngeal region. In those embryos in which chordal remains are present in the retropharyngeal region a close relation between such chordal remains and the pharyngeal bursa was noted. Embryo *O* has been added to show that the pharyngeal tonsil develops in connection with the pharyngeal bursa, if such bursa is present, and that the development of the pharyngeal bursa is independent of that of the pharyngeal tonsil, and not as Schwabach maintained, due to the development of the tonsil. Froriep and especially Linck have discussed the nature of the influence exerted by the notochord on the development of the pharyngeal

bursa, regarding its action as largely mechanical. Linck discusses this phase of the question at some length. The constant relation of the notochord to the pharyngeal epithelium, after the chorda begins to separate from the epithelium, the definite location of this area of contact, and the distinct reaction of the pharyngeal entoderm at the seat of contact leads me to regard this relation of chordal and pharyngeal epithelium other than an accidental one, and that thus to some extent the pharyngeal bursa may be regarded as a structure *sui generis*. In the early stages showing the anlage of the pharyngeal bursa the epithelium is distinctly thickened in the region of the future bursa, showing cell proliferation, although mitosis was not evident in my preparations. That a close connection between chordal and pharyngeal epithelium is necessary to the development of the pharyngeal bursa seems evident from the preparations of my series which show that the anlage of the bursa is present only when this connection between chordal and pharyngeal epithelium is maintained after the remainder of the notochord in the retropharyngeal region is separated from the pharyngeal epithelium. If in early stages of development, human embryos of from 10 mm. to 15 mm., crown breech length, this connection is so loose as to lead to a separation of the notochord from the pharyngeal epithelium throughout the entire retropharyngeal region, no pharyngeal bursa develops.

It is of interest to note finally that in the pig there is found a pharyngeal pocket which has been considered as the homologue of the pharyngeal bursa as found in man. Whether this pharyngeal pocket is a constant structure in the pig I am not prepared to say. Mrs. Gage and Mead have shown that in a certain per cent of pig embryos the head notochord reaches the retropharyngeal region and comes in contact with the epithelium of the pharyngeal vault. From Killian's comprehensive comparative studies we learn that a pharyngeal bursa, such as is found in man, is very rare, indeed found only in Arctomys marmota. In the mammals, the embryos of which are more generally accessible for laboratory work, only the pig has a pharyngeal bursa or its homologue, and only in pig embryos does the head notochord reach the retropharyngeal region and come in contact with the pharyngeal epithelium.

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DIMORPHISM IN THE SPERMATOZOA OF NECTURUS MACULOSUS

HELEN DEAN KING

From The Wistar Institute of Anatomy and Biology

SIX FIGURES

A study of the mature spermatocytes in the testes of Necturus maculosus has shown that these cells contain a peculiar multiple chromosome that divides unequally in the first maturation mitosis and thus leads to the formation of two classes of spermatozoa, one having more chromatin than the other. As a dimorphism in the spermatozoa of amphibians has not heretofore been reported, a brief description of the spermatocyte divisions in Necturus is given in the present paper: a more detailed account will appear when my investigation of the early growth stages of the spermatocytes has been completed.

In a very early prophase of division the primary spermatocytes contain a thick, apparently continuous spireme which is split longitudinally throughout its entire length. This spireme breaks into twelve segments of different lengths which condense rapidly to form the chromosomes for the first maturation spindle.

Two of the chromatin segments of the prophase are distinguished from the others on account of their greater length. One of these segments becomes a very large ring-shaped chromosome which is very conspicuous in the metaphase of the first mitosis when it is usually twisted in various ways (figs. 2, *L*; 3, *L*). In mitosis this chromosome divides equally, forming two *V*-shaped chromosomes which move to opposite poles of the spindle. The other long segment, as its later history shows, is not a bivalent but a multiple chromosome. In the late prophase this segment appears as a long, thick rod of nearly uniform diameter with the

two ends bent at nearly right angles to the main axis (fig. 1). The bent terminal sections are never of equal length, and the longer one is the 'accessory' or the '*X* chromosome' which became attached to one of the large bivalent chromosomes at an early period in the growth of the spermatocytes. Not infrequently, as shown in figure 1, *X*, the *X* chromosomes is detached from the main structure and connected with it only by linin.

The multiple chromosome goes into the first maturation spindle in practically the same form in which it appears in the late prophase, and in the metaphase it assumes such a position on the spindle that its main axis lies along one of the spindle fibers while the two bent terminal portions either project at right angles to the spindle (fig. 2), or they lie across the spindle fibers (fig. 3). There is more or less variation in the size of this chromosome in different spindles, due doubtless to fixation and staining, but in nearly all cases it extends over considerably more than half of the length of the spindle and in some cells it reaches from pole to pole. Sometimes this structure is of nearly uniform thickness throughout its entire length (fig. 3): more frequently, as shown in figure 2, it has slight constrictions that divide it into five parts and thus give it the appearance of a pentad structure. In its constricted form this chromosome appears remarkably like the multiple chromosome found by McClung ('05) in the spermatocytes of Hesperotettix speciosus and of various other species of orthoptera.

Whenever the multiple chromosome is shown in its entirety the portion of it which forms the *X* chromosome can readily be distinguished. Sometimes the *X* component seems to be an integral part of the multiple chromosome, although it invariably forms a sharp angle with the main axis of this structure (fig. 2, *X*). At other times, as shown in figure 3, the *X* chromosome is a short, rod shaped body lying close against one end of a large bivalent chromosome, but apparently not connected with it in any way.

The bent portion of the multiple chromosome at the end opposite to the *X* chromosome is usually much shorter than the *X* component (figs. 2 and 3), and as yet I have not found a single case in which this section was not directly continuous with the main axis of the multiple chromosome although always forming an angle

with it. It seems probable, from the evidence in hand at present, that this bent terminal portion is merely the end of one of the

All figures were drawn with the aid of a camera lucida under a magnification of about 1500 diameters: they have been reduced one-half. *L*, large ring-shaped chromosome; *S*, supernumerary chromosomes; *X*, *X* chromosome.

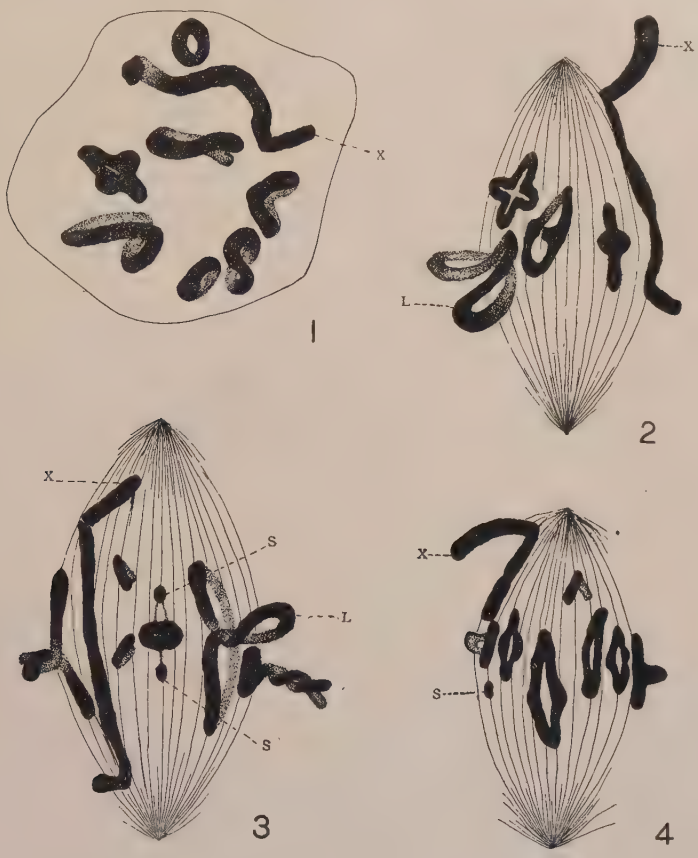

Fig. 1 Late prophase of the first maturation mitosis.
Figs. 2–4 Metaphases of the first maturation mitosis.

univalent chromosomes which helped to form the multiple chromosome during an early period in the development of the spermatocytes. There is the possibility, however, that this section is the small mate of the *X* chromosome, in which case the spermato-

cytes of Necturus contain an unequal pair of heterochromosomes instead of an *X* chromosome. This point cannot be cleared up until the early history of the spermatocytes has been studied.

When the first maturation mitosis occurs the multiple chromosome divides in such a way that one daughter cell gets one univalent chromosome and the *X* component (fig. 4) while the rest of the complex goes into the other cell.

The other ten segments of the prophase are considerably shorter than the two described above. At least six of these segments appear as small rings in the late prophase (fig. 1) and go into the spindle in this form. All of the remaining segments condense as crosses with one pair of arms considerably longer than the other (figs. 1 and 2). The cross-shaped chromosomes undergo considerable modification in form before the maturation division occurs. At first the longer axis of each of these chromosomes lies along one of the spindle fibers (fig. 2). This axis shortens gradually and its chromatin substance goes into the side arms which increase correspondingly in length. Just before division these chromosomes appear either as short, thick rods which lie across the spindle fibers, or as small elongated rings in which the central opening is a mere slit (fig. 3). In mitosis each of these chromosomes divides through its longitudinal axis, forming two small *V*-shaped chromosomes of equal size. A similar change in the shape of cross-shaped chromosomes preparatory to division has been found in the germ-cells of various other species of amphibians (Carnoy and Lebrun '99; Lebrun '01; King '05).

In connection with the change in the shape of the cross-shaped chromosomes in the spermatocytes of Necturus there sometimes occurs a process which, as far as I am aware, has not been observed to take place in the germ-cells of other amphibians in which chromosomes of a similar shape are found. A small mass of chromatin, at one or at both ends of the arms lying along the spindle fiber, becomes so firmly attached to the spindle fiber that it gradually becomes separated from the main body as the side arms increase in size. For a time such small fragments remain attached to the rest of the chromosome by linin-like strands (fig. 3), but eventually they break away from the parent mass and appear on the

spindle as small, round supernumerary chromosomes (fig. 4, *S*). These supernumeraries move with the *V*-shaped chromosomes to the spindle poles, and they can sometimes be distinguished in a late anaphase. These small chromatin masses are seemingly derived only from the chromosomes that appear on the spindle in the form of a cross, and as they move to the same pole of the spindle as do the univalent chromosomes to which they belong, their detachment from the main mass of chromatin does not lead to an unequal distribution of chromatin to the spindle poles. Supernumerary chromosomes are not found on all spindles and their formation is apparently due solely to the fact that at times the connection between the ends of the cross-shaped chromosomes and the spindle fibers is stronger than the force that is changing the shape of the chromosome and causing the chromatin to move into the side arms. I have not as yet been able to trace these small chromosomes beyond the first maturation spindle. Small supernumerary chromosomes have been found in the spermatocytes of various species of insects by Stevens ('12 a, '12 b) and by Wilson ('09), but they differ from the supernumerary chromosomes of Necturus in that they appear to be derived from the heterochromosomes and not from the ordinary bivalents.

The first maturation mitosis in Necturus is probably a segregation division, and there is seemingly a definite order in which the various chromosomes divide. The multiple chromosome divides after the division of one or of two of the smallest ring-shaped chromosomes (fig. 4); then follows the separation of the univalents which were united as crosses or as rings of medium size; lastly the large ring-shaped chromosome separates into its univalent components. Since the multiple chromosome divides at a very early period it is evident that the order in which the chromosomes divide is not dependent on the size of the chromosomes but on some other factor as yet undetermined.

There is a resting period of considerable length between the two maturation divisions, during which the chromosomes lose their visible identity and form a continuous spireme. The chromosomes that emerge from the spireme to go into the second maturation spindle are *V*-shaped structures that vary consider-

ably in size. As a rule two of these chromosomes are considerably larger than the others. One, with arms of equal length, is doubtless the univalent chromosome which with its homologue formed the large ring-shaped chromosome of the first maturation spindle. The other large *V*-shaped chromosome has arms that are slightly unequal. This is probably the chromosome derived from the multiple chromosome of the first spindle. Since the chromosomes are very much crowded together in the second spindle it becomes very difficult to follow the *X* chromosome. In most cases the *X* chromosome probably remains attached to one of the univalent chromosomes and divides longitudinally with it

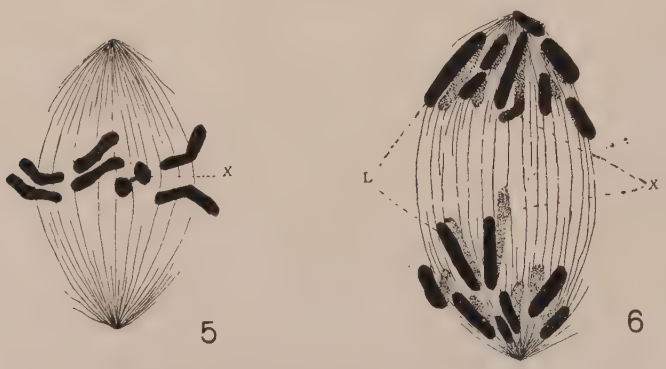

Fig. 5 Early anaphase of the second maturation mitosis.
Fig. 6 Late anaphase of the second maturation mitotis.

(fig. 6, *X*). At times, however, the *X* chromosome apparently breaks away from its attachment to the large chromosome and divides alone in the second mitosis, forming two small, nearly spherical chromatin masses one of which goes to each pole of the spindle (fig. 5).

Since the *X* chromosome passes undivided to one pole of the first maturation spindle and divides longitudinally in the second maturation mitosis two classes of spermatozoa are produced in Necturus, as in many other species of animals. Presumably both classes of spermatozoa are functional, as I have not found sufficient degeneration among the spermatids to warrant the assumption

that spermatocytes lacking the *X* chromosome degenerate, as is the case in Phylloxerans (Morgan '09) and several other forms.

The mature spermatocytes of Necturus show a condition of the *X* chromosome which is transitional between that found in the many species in which thé *X* chromosome can be traced as a separate structure throughout the development of the spermatocytes and the chromatin relations in such forms as the higher batrachians in which the *X* chromosome has apparently formed a permanent union with one of the large chromosomes and can no longer be distinguished by any method of technique at our command. It is hoped that the study of the early stages in the development of the spermatocytes will give further facts of interest regarding the relations of the *X* chromosome in this amphibian.

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ADULT HUMAN OVARIES WITH FOLLICLES CONTAINING SEVERAL OÖCYTES

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FOUR FIGURES

The two ovaries dealt with below were obtained at autopsy for histological purposes in this laboratory in the fall of 1909 from a negress eighteen years old. Macroscopically they appeared perfectly normal. There was no history taken of the case. When the sections were given out to the class it was found that many follicles contained more than one oöcyte. It was suggested by Professor Hardesty that it might be of interest to describe the conditions found and he kindly assigned the material and the problem to me.

The ovaries were fixed in Zenker's fluid, cut transversely in celloidin at 20 micra thick, mounted serially and stained with haematoxylin and congo red. The accompanying figures were made with the aid of the camera lucida. The epithelial lining of the Fallopian tubes showed satisfactory fixation, the cell boundaries and cilia being very well defined in the sections. The connective tissue in the ovaries appeared in normal proportion, the distribution of the follicles appeared normal, and the follicular epithelium was well fixed.

Follicles containing more than one oöcyte were found to be remarkably abundant. The greater number of these were in the earlier or smaller stages, varying from a follicular epithelium of one layer of cells to stages showing the beginning of the corona radiata. Of the larger follicles only, beginning with those far enough advanced to show the first appearance of liquor folliculi, one of the ovaries showed forty-five such follicles containing more than one oöcyte. The figures below represent an accurate count-

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ing of the oöcytes in each of all the follicles that were large enough to show a liquor folliculi developed:

The first ovary studied gave:

17	follicles	containing	2 oöcytes
6	follicles	containing	3 oöcytes
12	follicles	containing	4 oöcytes
7	follicles	containing	5 oöcytes
2	follicles	containing	7 oöcytes
1	follicle	containing	11 oöcytes

In the second ovary of the pair there were counted:

18	follicles	containing	2 oöcytes
4	follicles	containing	3 oöcytes
8	follicles	containing	4 oöcytes
5	follicles	containing	5 oöcytes
2	follicles	containing	6 oöcytes
2	follicles	containing	8 oöcytes
1	follicle	containing	9 oöcytes
2	follicles	containing	10 oöcytes
1	follicle	containing	13 oöcytes

Thus, in the two ovaries of the individual there was found a total of eighty-eight of the larger follicles containing more than one oöcyte. The counting of the oöcytes was confined to those sections of them in the series which contained the 'germinal spot' or nucleolus.

All of the sections of the first ovary studied were not available in series, a batch having been issued by the technician to the students for class work in organology before the peculiarity was observed. The section of the follicle represented in figure 1 may be taken as an example of the appearance of the polyovulation found.

The particular follicle represented in figure 1 contained eleven oöcytes measuring from 110 to 180 micra in diameter, with a total average diameter of 151 micra. This follicle was not spherical in the preparations. Measured transversely in three planes from the membrana propria of the stratum granulosum on the two sides, a mean diameter of 437 micra was found for it. Its longest diameter was found to be parallel with the long axis of the ovary and therefore had to be obtained by counting the sec-

tions in the series through which it appeared cut and multiplying by the known thickness of the sections. All of the oöcytes were attached to the stratum granulosum by means of cumuli oöphori. However, five of them in this case were attached at the same point by means of a common pedicle, appearing through the

Fig. 1 showing a polyovulated Graafian follicle which contained eleven oöcytes. The drawing is reconstructed as far as the oöcytes are concerned from a series of 12 sections each 20 micra in thickness. From the ovary of a negress of eighteen years. *C*, portion of a cluster of oöcytes; *MP*, membrana propria of stratum granulosum. × 57.

sections collected in a grape-cluster with a common cumulus, four being attached to the fifth and this attached in the usual manner. All of the eleven oöcytes in this follicle were normal in structure and were approximate in size to the diameters considered normal for this stage of the follicle.

The follicle represented in figure 2 is of another type of polyovulation found to be about equally common as that of figure 1. In this the oöcytes vary markedly in size, some being three times the diameter of others. This follicle was but approximately half the size of that shown in figure 1. It contained, through the series of sections, thirteen oöcytes of which five were large, averaging 150 micra in diameter, the remaining eight being smaller. Three of them were especially small, not measuring above 50 micra in diameter. All the thirteen oöcytes here were attached

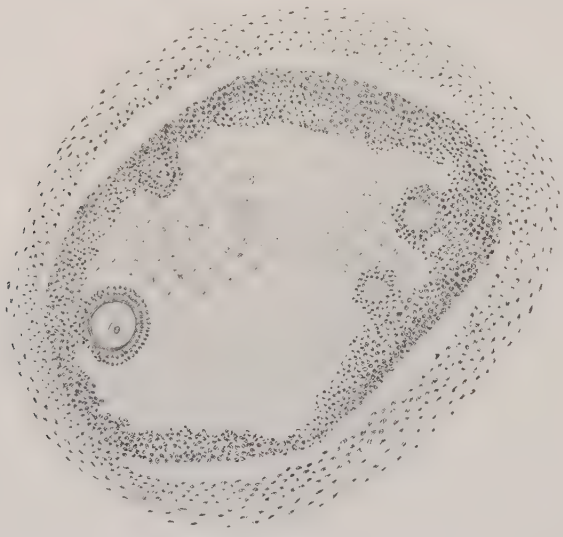

Fig. 2 A section of a polyovulated Graafian follicle from the same individual as figure 1. Shows a type in which the oöcytes vary in size. × 57.

separately to the stratum granulosum by pedicles varying but slightly in width and with cumuli oöphori in about equal amount.

In studying the smaller follicles, most of which were situated nearer the periphery of the ovary, it was observed that a large number of those containing more than one oöcyte presented the gradation shown in figure 3. This figure was drawn to represent a smaller follicle, *A*, not yet developing a liquor folliculi and in which the oöcytes were of the same size, and to represent, *B*,

a smaller follicle with liquor folliculi beginning to form and with one oöcyte larger than the others. These conditions were observed in a large number of such follicles and polyovulated follicles of similar sizes were much more numerous than the larger sizes represented in the previous figures.

The staining reaction appeared the same in all respects in both the large and the small oöcytes, the histological picture showing no indication of degeneration in any case observed beyond the variations in size.

There were quite a number of polyovulated follicles about as large as the one shown in figure 1 which were found to contain from two to six oöcytes of large size and of perfectly normal appearance. The largest follicle observed in either of the ovaries

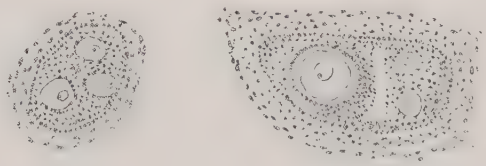

Fig. 3 Drawing, showing sections of two smaller polyovulated Graafian follicles from same source as figures 1 and 2. *A*, a smaller follicle with oöcytes of equal size; *B*, a smaller follicle with oöcytes of varying size. × 57.

gave an average diameter of 633 micra. This contained only one oöcyte whose measurements gave a diameter of 201 micra. There were two other follicles both larger than the one shown in figure 1, each of which contained but one oöcyte. Thus it may be seen that the polyovulated follicles did not comprise the largest follicles.

Lying in the peripheral stroma of both the ovaries, mostly subjacent to the tunica albuginea, were the numerous primitive follicles found in all individuals. These follicles for the most part showed the usual one layer of low epithelial cells enclosing an oöcyte of the smallest size (*A* and *PF*, fig. 4). Occasionally, near the periphery, several of these small oöcytes appeared closely associated with each other, unevenly surrounded by an irregular

line of dispersed nuclei which no doubt represented follicular epithelium in single layer.

Another interesting and seemingly unusual observation could be made in the sections of these adult human ovaries. The germinal epithelium (*GE*, fig. 4) appeared to be still proliferating and showed quite a number downfoldings or invaginations into

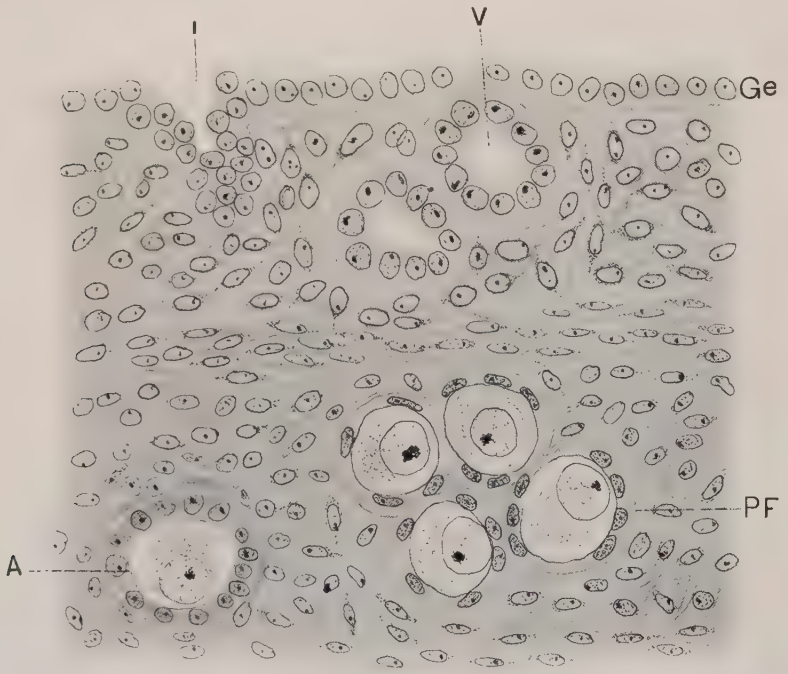

Fig. 4 Drawing, showing vertical section of superficial area of ovary used for previous figure. *Ge*, Germinal epithelim; *I*, invagination or down cropping of germinal epithelium; *V*, vesicle or follicle without oöcyte; *PF*, primary or resting follicles; *A*, primary follicle beginning further development. × 280.

the stroma below. One stage of such invagination is indicated in figure 4, *I*. Many stages of this could be found, including stages in which the downcroppings of the epithelium was partially and also wholly constricted from its continuation with the superficial epithelial covering, giving the appearance of short, wide tubules in the immediately underlying stroma. In no case, however,

did any of the cells of these downcroppings, or tubules, show any sign of differentiation into primitive oöcytes. Further, follicles, or rather vesicles, could be often observed consisting of a wall of a simple layer of cells of the size of those comprising the germinal or superficial epithelium, and in every way suggesting recent formation, but, in no case did any of these follicles contain a vestige of an oöcyte. They merely enclosed a cell-free space as shown in figure 4, *V*. A theca folliculi about these vesicles was no more definitely formed than about the ordinary primitive follicles.

If, as is generally believed, the invagination of the germinal epithelium with the differentiation of certain cells into oöcytes and the formation of 'egg tubes,' 'egg nests,' and primitive follicles, terminates before or shortly after birth, or certainly before the eighteenth year, then such a downcropping and evidently resultant vesicles as seen here must represent a period in which follicles are formed but not oöcytes. None of these empty vesicles were found of larger size nor were any observed indicating increase in size by further growth.

It is not the intent of this paper to try to explain the occurrence of the several oöcytes in a single follicle. The phenomenon seems to be considered one of very rare occurrence. The first notice I have found of such in the literature is that of Waldeyer in 1870. His illustration, from the ovary of the dog, is given in Huber's revision of Böhm and Von Davidoff's Histology ('00) and is also copied by Schafer in the latest edition of Quain's Anatomy, volume 2, part 1, 1912.

Van Beneden ('80) observed in the ovary of the 'horse-shoe bat' follicles containing two or three, and in one case a follicle with four oöcytes. These oöcytes were of varying size and none of the follicles containing them were advanced to the stage showing liquor folliculi or follicular cavities. Schöttlander ('93), working with human material, all of which was admittedly pathological, describes numerous small follicles containing two or three oöcytes. Practically all in this condition possessed a follicular epithelium of but one layer of cells and these were of the primitive type. All were in ovaries of foetuses of varying ages and at term. He

described some follicles, somewhat more advanced, in which there appeared a large but abnormal oöcyte in the center of the follicle while certain cells of the follicular epithelium appeared enlarged as though differentiating into öocytes. These latter he called 'accessory ova.' He considered all the cases of polyovulation observed by him as degenerative conditions.

Stoeckel ('98), also working with human material, found in the ovary of a woman of twenty-nine years, several follicles containing two or more oöcytes. This is the only reference I have found in the literature in which polyovulation has been observed in adult human material. Bouin ('00), reports finding in the ovary of the dog follicles containing two, and some, three, oöcytes. He describes one follicle in which he estimates the presence of ten oöcytes. All these, he describes as perfectly normal in appearance, the latter case being a larger follicle with perfectly normal stratum granulosum, and none of them showed any sign of pyknosis or other degenerative changes.

Honoré ('00), working with ovaries of the rabbit, describes a few follicles containing more than one oöcyte and one containing as many as four. These oöcytes were of varying size in the follicle, and the follicles were of the smaller stages. The follicle containing four oöcytes showed one in its center while the other three were much smaller and were buried among the cells of the follicular epithelium, similar to those described by Shöttlander. He describes these smaller buried oöcytes as undergoing atrophy. He cites Nagel and Rabl as having observed polyovulation but does not state the animals in which they found it and I have not been able to consult their papers.

The Bouins ('00) described a follicle in the ovary of the adult bitch which contained eleven oöcytes. These were very varied in size and position, the smaller being buried among the cells of the follicular epithelium as was observed by Honoré.

As to the origin or cause of such polyovulation, Bouin and Honoré considered these several oöcytes as having been imprisoned in the same connective tissue theca at the time of the closing off or constriction of the egg tubes or egg nests into separate follicles.

Waldeyer thought that, in the polyovulation observed by him, the additional oöcytes arose by mitotic division of the oöcyte previously existing in the follicle, and Nagel, quoted by Honoré, seems to have supported Waldeyer in this view.

Stoeckel held that polyovulation results from the cytoplasmic division in a follicle of oöcytes originally containing two or three nuclei, and that the separate oöcytes thus arising become dispersed in the follicle by the subsequent proliferation of the cells of the follicular epithelium.

Rabl, quoted by Honoré, held that the follicular epithelium encloses more than one oöcyte from the beginning of its formation. This may mean that from the stage commonly known as that of the 'egg nests of Pflüger,' two or more closely associated oöcytes may become surrounded by a common follicular epithelium and thus remain. He believed that polyovulated follicles are subsequently separated into monovulated follicles by the proliferation and ingrowth of the follicular epithelium, followed by the ingrowth and development of the theca folliculi. This view, however, can apply only to the younger stages of follicular development and not to the conditions found in larger follicles as observed in this paper. Honoré himself seems unable to arrive at a definite explanation of polyovulation, but is inclined to support the views of Rabl.

In the adult human ovary here mentioned, the tissues in all respects seemed normal. No evidences of degeneration were observed and the material was well fixed. The only peculiarities noticeable were the abundant instances of polyovulation and the evident downcropping at this age of the germinal epithelium and the formation of vesicles or follicles not containing oöcytes. No evidence of cell division, nuclear or cytoplasmic, were noticed in any of the oöcytes. Therefore it is improbable that the views advanced by Waldeyer, supported by Nagel, and that advanced by Stoeckel are applicable in this case. The fact that in about one-half of the polyovulated follicles studied here certain of the oöcytes were much smaller than others may indicate that these smaller oöcytes have been arrested in their development. Very probably such would degenerate during the further advancement

of the process of ovogenesis. Possibly all would disappear except one. Or, possibly all such follicles would undergo *atresia* and thus never reach the stage of extrusion of the ovum.

In the series of sections of these ovaries, there may be seen in the stroma immediately underlying the tunica albuginea and among the primitive or resting follicles, series of smaller oöcytes closely associated in lines of three or four, an entire line being bounded about by a single line of nuclei of follicular cells but with no follicular cells between the oöcytes. Again, small clusters of oöcytes appear in which certain of the oöcytes seem to lack a separately distinct follicle. This condition is partially indicated with two of the oöcytes shown in figure 4, *PF*. It is therefore, possible that some of the polyovulated follicles of the advanced stages may result from the development of a common follicular epithelium about two or more oöcytes.

Of course, nothing definite may be advanced as to whether two or more oöcytes in a follicle ever proceed in their development though the stages of maturation and thence result in an expulsion of two or more ripe ova from a single follicle.

I wish to acknowledge my indebtedness to Professor Hardesty for his suggestions and guidance in the preparation of this paper.

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ON THE TELEOSTEAN FOREBRAIN

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From The Institute of Anatomy, University of Minnesota

THREE FIGURES

In the Journal of Comparative Neurology for June, 1912, appears a paper on the olfactory centers of teleosts by Dr. Sheldon, from Professor Herrick's laboratory in the University of Chicago. This paper was submitted for publication just before that by the writer ('11 b) which appeared in the same Journal for December, 1911. Dr. Sheldon and the writer carefully examined each other's manuscripts in July, 1911. Dr. Sheldon's paper presents the results of very careful study of a large number of specimens of the carp brain prepared by the most approved methods. It is the object of this note to point out the agreement as to facts in the two papers mentioned, and to comment briefly on the differences of interpretation of those facts.

The following table will indicate at once the corresponding structures as described in the two papers:

Johnston	*Sheldon*
Bulbus olfactorius	Formatio bulbaris and nucleus olfactorius anterior
Nucleus olfactorius medialis	Corpus praecommissurale Nucleus medianus Pars commissuralis Pars supra-commissuralis Pars intermedia
Nucleus olfactorius lateralis	[Lateral part of the nucleus medianus of the corpus praecommissurale.]
Primordium hippocampi	Nucleus olfactorius dorsalis Nucleus olfactorius lateralis Lobus pyriformis Nucleus taeniae Palaeostriatum

Johnston	*Sheldon*
Lobus pyriformis	[Lateral part of nucleus medianus; plus the nucleus commissuralis lateralis?]
Somatic area	Nucleus entopeduncularis and diffuse cells about it and perhaps the nucleus commissuralis lateralis.
Nucleus praeopticus	Nucleus praeopticus parvocellularis. The nucleus magnocellularis seems to be a special development in teleosts.

It is well known that there are in the adult teleost brain a much larger number of discrete nuclei and fiber bundles than are seen in the brains of other fishes. This gives the appearance of a high degree of specialization. Dr. Sheldon's description of the adult brain of a single teleost shows these numerous discrete gray masses and fiber bundles, while the writer's description, based upon several ganoids and young teleosts, seems at first sight to differ greatly because of the small number of centers and tracts described. That this is the chief difference between the descriptions given in the two papers will appear from an examination of the several structures seriatim.

In the olfactory bulb most authors recognize a nucleus olfactorius anterior. While I have described the same gray matter under the name of deep cells (stellate cells, granules, etc.), I have insisted that these belong to the formatio bulbaris because their dendrites come into relation with olfactory fibers in the glomeruli and their neurites proceed as fibers of the olfactory tract to the secondary olfactory centers. Sheldon fully and clearly confirms this relation to the olfactory fibers. He describes also a tractus olfactorius ascendens arising in the precommissural body and ending in relation with these deep cells. Sheldon speaks of the nucleus olfactorius anterior as secondary olfactory tissue but his description does not show that any olfactory tract fibers end in it.

The medial olfactory nucleus or precommissural body presents practically the same condition in the forms studied by Dr. Sheldon and myself. The 'pars intermedia' of Sheldon's description which connects the precommissural body with the central gray of the thalamus, is less conspicuous in ganoids but is present. It should be noticed, however, that Dr. Sheldon seems to include in the nucleus medianus of the precommissural body not only the

anterior part of the medial olfactory nucleus but also the lateral olfactory nucleus of my description. This is a serious difference of interpretation, the significance of which will be referred to later.

In the writer's description the large dorsal part of the lateral lobe of the forebrain is given the name primordium hippocampi. The grounds for this are that this area is characterized by peculiar pyramidal cells with spiny dendrites, receives olfactory fibers of the second and perhaps of the third order, and receives ascending fibers from the hypothalamus which are believed to be gustatory in function. The cells of the peculiar type described are present throughout all parts of the area in question and the ascending fibers from the hypothalamus spread uniformly throughout the whole area in ganoids and young teleosts. The primordium is bounded medially by the sulcus limitans hippocampi in the ventricular surface and laterally by the external sulcus and the lateral olfactory tract. In the substance of the lateral lobe there is always visible a more or less conspicuous and extensive cell-free zona limitans which separates this area from the medial and lateral olfactory nuclei.

In the adult carp Sheldon describes in the corresponding area several more or less distinct gray masses and details the fiber tracts connected with each one. The boundaries of the area as a whole agree with those given by the writer. The ventricular sulcus is given the name fissura limitans telencephali. In this area Sheldon recognizes a nucleus olfactorius dorsalis occupying the dorso-medial angle of the everted lateral lobe and adjoining the precommissural body; a nucleus olfactorius lateralis which includes the greater part of the entire structure; a lobus pyriformis and a nucleus taeniae adjacent to the taenia fornicis in the caudal part of the lateral lobe; and a palaeostriatum deeply imbedded in the lateral lobe, beneath the nucleus olfactorius lateralis. Dr. Sheldon's figures 32, 33, 45, 48, 49, 50, 51, 52, 53, 58, 59, 60, show the cells with the peculiar spiny or thorny dendrites in all the nuclei mentioned except in the nucleus olfactorius dorsalis. In the text (p. 193) it is stated that many of the cells of this nucleus "resemble the dorsal cells of the nucleus olfactorius lateralis (figs. 48, 49, 56)." So far as the types of cells are concerned, all

the nuclei mentioned might well be regarded as parts of one large center. This is a very welcome confirmation of the writer's description of the primordium hippocampi in fishes, because the existence of these cells of a peculiar type in the primordium hippocampi of various fishes and amphibians has been used as one of the chief means of identifying the primordium hippocampi in cyclostomes ('02 a, '06, and '12 b[1]).

With regard to the various fiber tracts connected with these several nuclei, there is noticeable a close similarity between all parts of the area which I have called primordium hippocampi. All parts receive olfactory tract fibers, those from the medial tract entering the medial border those from the lateral tract entering the lateral border. There are decussating fibers also entering the lateral border. Sheldon has described further tertiary olfactory fibers from the precommissural body entering the nucleus olfactorius dorsalis and the palaeostriatum. Although such tertiary fibers are probably present in cyclostomes ('12 b), they are proportionately few in selachians and have not been certainly demonstrated heretofore in teleosts. It can not be argued that the term primordium hippocampi must be limited to that part of the forebrain into which these tertiary olfactory fibers enter.

All the nuclei mentioned, except the dorsal olfactory nucleus, receive ascending fibers from the hypothalamus by way of the tractus olfacto-hypothalamicus lateralis of Kappers or by way of the tractus strio-thalamicus. All of these ascending fibers evidently correspond to the tractus pallii of the writer in ganoids and young teleosts. In several of the forms studied by the writer (Polyodon, Ameiurus) there are large bundles of the tractus pallii rising into the caudal part of the primordium hippocampi. These would correspond to the ascending fibers in the tractus olfacto-hypothalamicus lateralis, although they are less completely segregated than in the carp. The ascending fibers in the tractus strio-thalamicus in the carp have a distribution similar to that of the larger part of the tractus pallii in the forms described by the writer. It is to be noted that ascending fibers from the hypo-

[1] Johnston, The telencephalon in Cyclostomes, Jour. Comp. Neur., vol. 22, August, 1912.

thalamus running in the medial forebrain bundle go up to the medial border of the primordium hippocampi in the sturgeon, partly direct and partly after crossing in the anterior commissure. Sheldon traces similar fibers only as far as the precommissural body.

Descending fibers are described by Sheldon from all these nuclei to the hypothalamus by way of the medial or lateral hypothalamic tract or the tractus strio-thalamicus. This confirms the early description by Van Gehuchten and is in agreement with the writer's findings in the small number of brains of Amia in which such fibers were impregnated. The unequivocal description by Sheldon removes all doubt as to the descending pathway from the primordium hippocampi in teleosts. This is also in agreement with the conclusion reached by several authors that the tractus pallii in selachians is composed of both ascending and descending fibers.

Finally, a tract called the tractus taeniae arises from the nucleus taeniae and runs to the nucleus habenulae. This is said to be the morphological equivalent of the tractus cortico-habenularis lateralis of Herrick in the frog. It is possible that this part is accompanied by a few commissural fibers constituting a posterior pallial commissure. This tractus taeniae of Sheldon together with his somewhat hypothetical posterior pallial commissure corresponds to what the writer has termed the posterior pallial commissure. Although a true commissure has not been certainly demonstrated by either of us, the presence of such a commissure in cyclostomes (Johnston '12 b), selachians, amphibians and reptiles, renders its existence in ganoids and teleosts very probable.

Both the medial and lateral borders of the general area are connected by commissures which Sheldon calls the anterior and posterior hippocampal commissures. In Amia there is a single large commissure distributed to the whole area.

There is thus seen to be a close agreement as to facts in the description of the general area under consideration. Dr. Sheldon, however, interprets this general area as including the lateral olfactory nucleus, the pyriform lobe, the nucleus taeniae and a palaeostriatum, while only the nucleus olfactorius dorsalis is

regarded as the primordium hippocampi. Since the whole area has so nearly identical structure and connections throughout its parts, this interpretation seems to the writer untenable.

First, the commissures, the habenular and the hypothalamic connections all point more clearly to the lateral part of this area as the homologue of the primordium hippocampi in cyclostomes, selachians and amphibians. It is only in the entrance of tertiary olfactory fibers that Sheldon's dorsal olfactory nucleus bears a special resemblance to hippocampus. This is the least important characteristic of the hippocampal primordium in lower forms and in the carp it is shared with the palaeostriatum.

Second, most writers on the teleost brain in identifying the lateral olfactory nucleus have ignored the important fact of eversion. They regard the structures which are laterally placed as morphologically lateral. The position of the line of attachment of the tela chorioidea, the form of the lateral lobes, the position of the zona limitans, all show that the so-called lateral olfactory nucleus is not lateral but ventricular and that its extreme ventro-lateral border where the tela is attached is morphologically the dorsal border of the lateral brain wall. The lateral olfactory nucleus of Sheldon and especially the lobus pyriformis and nucleus taeniae, so far from being morphologically lateral are adjacent to the taenia fornicis and correspond to the dorso-medial border of the hemisphere in evaginated brains like that of the frog.

Third, the reason for thus ignoring the significance of eversion is found in the fact that the lateral olfactory tract is largely distributed to the everted ventricular region. This is therefore given the name of lateral olfactory nucleus. Now when the lateral olfactory nucleus in all other vertebrates is examined it is found that it never has the tela chorioidea attached to it and that it never is removed from the external surface of the brain and restricted to the ventricular surface. In all other vertebrates the lateral olfactory nucleus is separated from the tela chorioidea by at least the primordium hippocampi and it occupies a part of the true lateral or latero-basal surface of the telencephalon. The structure which Edinger, Goldstein, Kappers, Sheldon and others call the lateral olfactory nucleus in teleosts is a great mass oc-

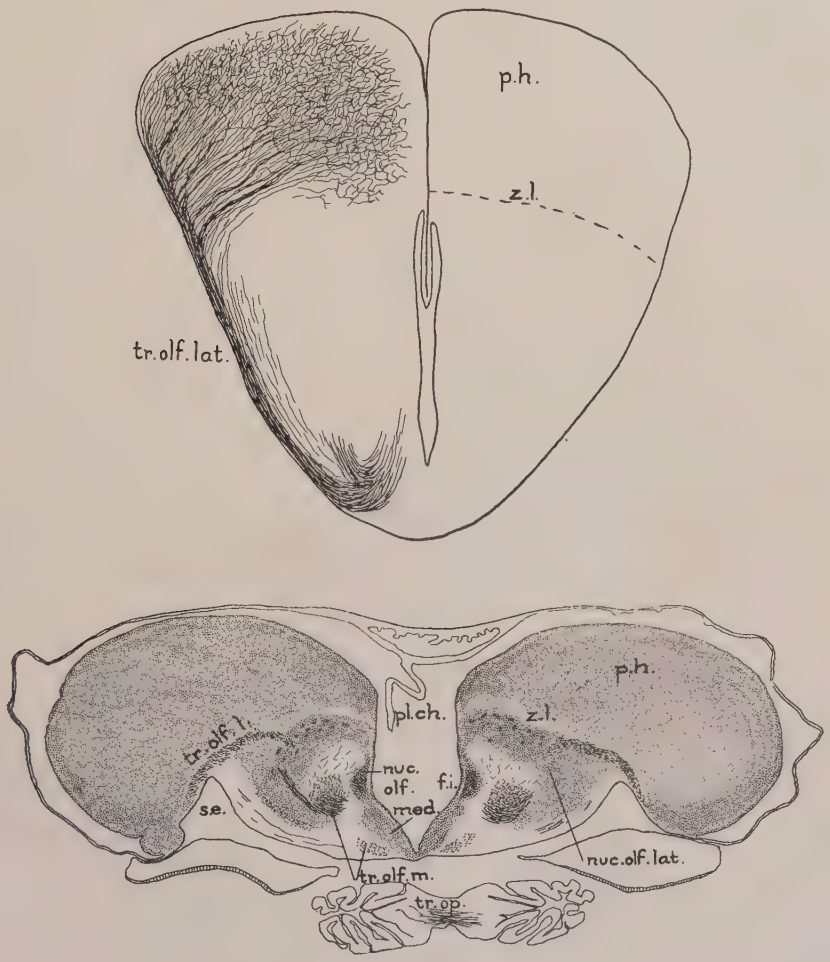

Fig. 1 Two figures for comparison of the transverse section of the telencephalon in front of the anterior commissure in a 15 mm. Amia (above) and in the adult. These figures show the actual change of form involved in eversion. It is the region dorsal to the zona limitans which is rolled and pushed outward, chiefly because of its own increase in volume. The same cause leads to the formation of the ventricular sulci. *f. i.*, groove leading to the interventricular foramen: *nuc. olf. med.* and *nuc. olf. lat.*, medial and lateral olfactory nuclei; *p.h.*, primordium hippocampi; *s.e.*, sulcus externus; *tr. olf. l.* and *tr. olf. m.*, lateral and medial olfactory tracts; *z.l.*, zona limitans.

cupying the ventricular surface and reaching the external surface only at its taenia-border.

The behavior of the lateral olfactory tract, however, does not compel us to identify this structure with the lateral olfactory nucleus, for two reasons. The lateral olfactory tract in selachians ends in large part in the primordium hippocampi, and in teleosts and ganoids a large part of the olfactory tract fibers

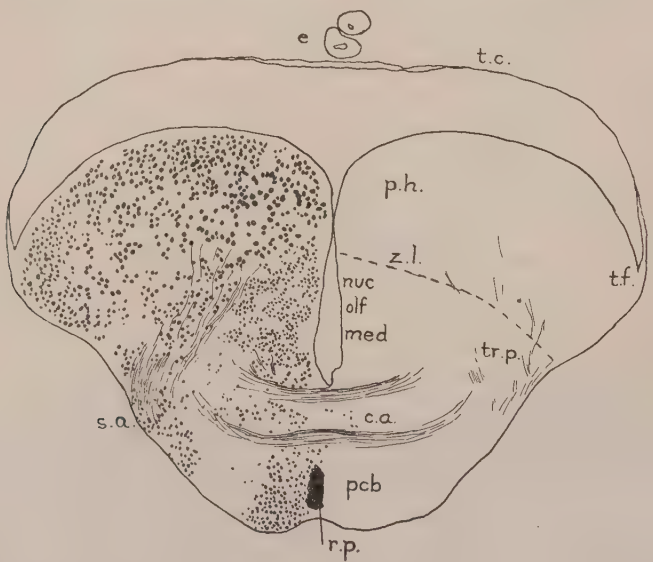

Fig. 2 A transverse section of the brain of Ameiurus of 19 mm., at the level of the anterior commissure. There is already slight eversion here as compared with the 15 mm. Amia and there is a broad shallow sulcus externus. Comparison of this with Sheldon's figures 38 and 56 will show that eversion in the teleost takes place in the same way as in Amia. *c.a.*, anterior commissure; *p.c.b.*, precommissural body; *r.p.*, preoptic recess; *s.a.*, somatic nucleus; *t.f.*, taenia fornicis; *tr.p.*, tractus pallii.

which enter the so-called lateral olfactory nucleus come from the medial olfactory tract. On the other hand, in ganoids and teleosts a large part of the lateral olfactory tract ends in the latero-basal superficial area below the so-called sulcus or fovea endorhinalis. It is this area which really occupies the lateral surface of the forebrain as in all other vertebrates, which the writer believes is the lateral olfactory nucleus.

In all vertebrates the lateral olfactory nucleus is separated from the primordium pallii or pars pallialis hemisphaerii by a more or less conspicuous sulcus in the external surface of the brain. In petromyzonts this is the deep obliquely placed groove seen in the dorsal aspect between the roof of the evaginated hemisphere and the elevated wall of the telencephalon medium, which has been shown by the writer to be the homologue of the primordium hippocampi of other vertebrates. In selachians the primordium hippocampi is evaginated into the roof of the hemisphere, but is marked off from the lateral olfactory nucleus by a groove and a narrow cell-free zona limitans in the lateral wall. This zona limitans hippocampi extends along the lateral wall, over the olfactory peduncle and around the rostral wall to meet its fellow in the medial wall at the neuroporic recess. The parts of the wall below this zona limitans include the lateral and medial olfactory nuclei, tuberculum olfactorium, etc. In amphibians a similar zona limitans separates the pars pallialis from the pars sub-pallialis as Gaupp has shown. In reptiles and mammals and possibly even in amphibians the general or somatic cortex has pushed in between the lateral olfactory nucleus and the primordium hippocampi, so that the zona limitans, or the sulcus which marks it externally, becomes the dividing line between the lateral olfactory nucleus and the general cortex. This groove in mammals is the fissura rhinalis which bounds laterally the lateral olfactory nucleus and the pyriform lobe.

Earlier authors (C. L. and C. J. Herrick '91, Edinger '96, Goldstein '05, Kappers '06) compared the groove on the latero-basal aspect of the teleost brain with the fissura rhinalis of mammals. Goldstein ('05, p. 143) cites Edinger's view that this is one of the most important brain fissures and that the pallium is always to be found just lateral to it. Kappers and Theunissen ('08) present a new view, namely, that the teleostean sulcus represents the sulcus endorhinalis,—"weil sie innerhalb des secundären Riechgebietes liegt." This is the view now taken by C. J. Herrick and Sheldon. The difficulty with this view is that the sulcus in fishes does *not* lie within the secondary olfactory area. That the area above it, which is now under consideration, does receive secondary olfactory

fibers is perfectly true. But so does the hippocampal formation throughout the vertebrate series. What has escaped the attention of these recent authors is the fact that the entrance of a large bundle of ascending fibers from the hypothalamus makes of this body something fundamentally different from the simple secondary olfactory centers. This has been fully set forth on page 525 of my recent paper on the forebrain of ganoids and teleosts.

Morphologically, however, it is clear that the sulcus endorhinalis of mammals does not correspond to the sulcus in question in fishes. The sulcus endorhinalis lies within the basal olfactory centers, between the lateral olfactory tract and the olfactory tubercle, and rostrad *ends upon the ventral surface of the olfactory peduncle*. In all fishes, amphibians and reptiles, the groove here under consideration extends along the lateral surface of the hemisphere, rises dorsally as it goes rostrad and *passes over the olfactory peduncle or olfactory bulb*. In this disposition the groove agrees with the fissura rhinalis of the mammalian brain and differs conspicuously from the sulcus endorhinalis. The writer is therefore convinced that the earlier view of Edinger, Kappers and Goldstein was correct.

In my recent paper I gave to the broad groove in the teleost forebrain the descriptive name sulcus externus. This was done because I do not regard this broad groove as actually homologous with anything in the brains of other vertebrates. A zona limitans is usually recognizable in ganoids and teleosts and this is homologous with the zona limitans lateralis in selachians and amphibians. The *sulcus* limitans lateralis in selachians and amphibians is a very slight groove not comparable in size and form with the sulcus externus of the teleost brain. Moreover, this sulcus externus is not present in the young stages of either ganoids or teleosts. It appears only in later stages as the result of the process of eversion. It is formed by the actual folding outward of the whole thickness of the dorsal part of the lateral forebrain wall. It is only the line along which the cell-free zona limitans in the substance of the lateral lobe meets the lateral surface that can be compared with the sulcus limitans lateralis in selachians and amphib-

ians. This line lies somewhere within the broad sulcus externus, but there appears to be considerable variation as to the form and position of this sulcus or fovea in adult ganoids and teleosts.

In the relation of the lateral olfactory tract to this sulcus there is further evidence for the homology of the sulcus limitans lateralis with the fissura rhinalis. The lateral olfactory tract runs longitudinally somewhat parallel with this sulcus, many of its fibers turning up into the primordium hippocampi. When the eversion of the brain wall is taken into account it is seen that the morphological position of the lateral tract is medio-basal to the sulcus. This is clear from Goldstein's figure 4 and Sheldon's figure 24, and is still more clear in the simpler ganoids and in young ganoids and teleosts. In Acipenser of 30 cm. where the eversion is slight, the lateral olfactory tract lies wholly basal to the zona limitans. In adult Amia ('11 b, figs. 5, 6) the lateral olfactory tract lies partly in the zona limitans and partly basal to it. In Polyodon ('11 b, figs. 55, 56) the lateral tract evidently lies wholly medio-basal to the zona limitans. The same condition prevails in the selachians; and in mammals, where the somatic cortex has pushed in between the olfactory tract and the hippocampus, the lateral tract lies in the same relation to the fissura rhinalis as it holds to the zona limitans lateralis in the fishes.

The above considerations have led the writer to the conclusion that the area seen from the ventricular surface in teleosts between the sulcus limitans hippocampi and the line of attachment of the tela chorioidea is the primordium hippocampi as it is in cyclostomes and selachians (and also in amphibians and reptiles except that the somatic cortex pushes into this area). The entire region has essentially the same structure and fiber connections throughout, although in adult teleosts the great increase in volume is accompanied by eversion and by the formation of new sulci such as the sulcus ypsiliformis. This has been accompanied by the segregation of gray masses and fiber bundles which Sheldon and others have described as distinct centers and tracts.

This view carries with it the interpretation of the lateral superficial part of the olfactory lobe rostral to the anterior commissure

as the lateral olfactory nucleus. This area is included by Sheldon in his nucleus medianus septi, but it must be observed that it belongs to the lateral wall of the brain and has nothing to do with the septal region. In ganoids it is largely separted from the nucleus medianus septi by that part of the olfactory bulb which is called nucleus olfactorius anterior ('11 b, figs. 5, 70). This true lateral olfactory nucleus extends caudad only about to the level of the anterior commissure where it meets with the somatic area. In selachians a well developed correlating tract has been found between the two centers and in higher vertebrates a part of this somatic area doubtless remains in connection with the lateral olfactory nucleus to constitute the pyriform lobe.

Dr. Sheldon locates the lobus pyriformis in the extreme lateral part of the lateral lobe to which the tela chorioidea is attached. Upon the view of eversion of the teleost forebrain held by Mrs. Gage, Kappers, the writer and others, this part of the lateral lobe corresponds to the medio-dorsal border in evaginated brains. The region which Sheldon has called the primordium hippocampi, on the other hand, corresponds to a portion of the lateral wall of the evaginated brains. This is illustrated in figure 3 which shows at a glance how untenable this interpretation is. Dr. Sheldon has seen the weakness of these homologies and has suggested that the form of the teleost forebrain is due not to a process of eversion such as Mrs. Gage set forth but to a wandering and shifting of the relative position of the gray masses (Sheldon '12, pp. 244–245). No facts are given upon which this hypothesis of a modified eversion is based and the writer has seen nothing in his study of larval stages of ganoids and teleosts to give support to it. The process of eversion in teleosts seems to the writer to consist of a turning outward together with some plastic pushing or shifting laterad of the whole mass of the lateral wall dorsal to the zona limitans. The turning outward carries with it the attachment of the tela chorioidea; and the dorsal border—taenia fornicis—in ganoids and teleosts is the same as in all other vertebrates, but is carried far latero-ventrally by eversion. The writer fails to see any ground upon which we may expect to find the lobus pyriformis in any vertebrate located adjacent to the taenia fornicis.

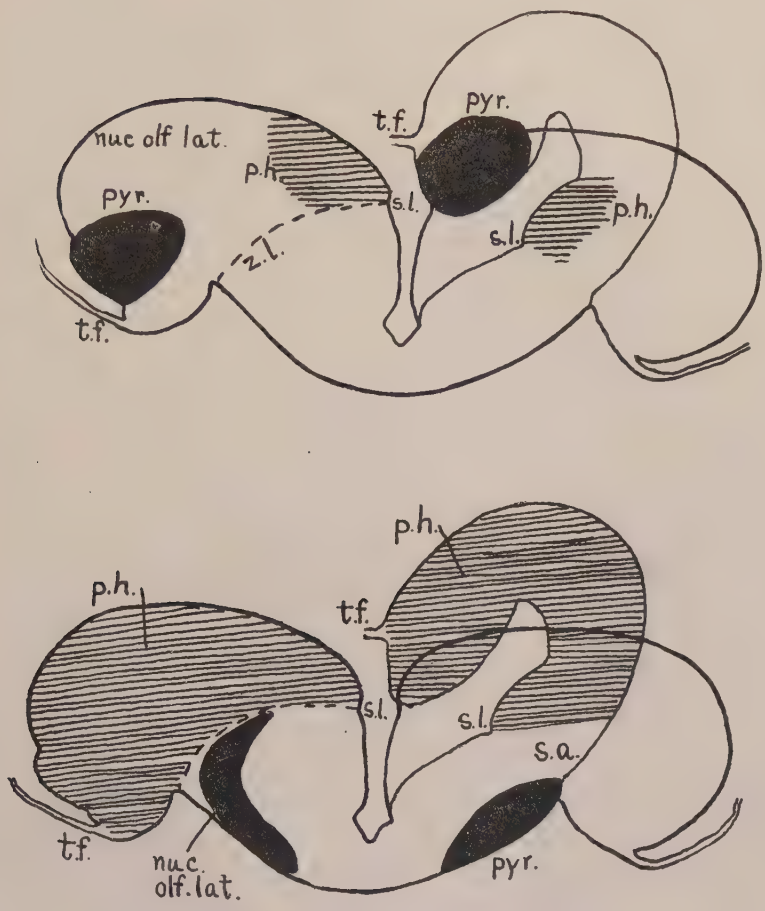

Fig. 3 Two diagrams based upon transverse sections at the level of the anterior commissure of Amia, to illustrate the conceptions of the relations of the primordium hippocampi held by Dr. Sheldon and by the writer. In both diagrams the form of cross section of the hemisphere of an amphibian is superimposed upon the right half of the Amia section. On the left in the upper diagram the position of the pyriform lobe and primordium hippocampi is shown as Sheldon has located them. On the right the position of these centers is shown as they would come to lie in a brain which was evaginated. In the lower diagram the position of these centers is shown as they are understood by the writer, *pyr.*, pyriform lobe; *s.l.*, sulcus limitans hippocampi. *s.a.* in this figure indicates the region where the somatic nucleus pushes between the basal olfactory centers and the primordium hippocampi to give rise to the general cortex.

As to the homology of the nucleus olfactorius dorsalis of the carp with the primordium hippocampi of the frog, Sheldon admits that there are few points of resemblance (p. 246). He concludes (p. 247):

> The materials found in the amphibian primordium hippocampi are not completely separated in the teleosts from the other elements of the secondary olfactory nucleus, being represented chiefly in the nucleus olfactorius dorsalis or primordium hippocampi and to a less degree perhaps in the nucleus olfactorius lateralis and nucleus pyriformis.

This approaches closely the view of the present writer. If this whole structure dorsal to the zona limitans is considered to be primordium hippocampi, its resemblance to that of the frog and selachians, in position, structure and connections, is clear.

In this connection Sheldon compares his sulcus limitans telencephali with the fissura limitans hippocampi of Herrick in the frog (fissura arcuata of Gaupp), although he states that the homology is incomplete. Indeed it is difficult to see what could lead the author to make any comparison between the two. The teleostean sulcus limitans telencephali lies in the ventricular surface of the *lateral* brain wall, while the fissura limitans hippocampi of Herrick lies in the *medial* wall of evaginated brains. The teleostean sulcus clearly is identical with the sulcus limitans lateralis of the frog's brain, and a fissura limitans hippocampi (Herrick) cannot be present or be conceived of in an unevaginated or everted brain. Such a sulcus can be present only in those forms in which evagination has carried a part of the medial olfactory nucleus out into the medial wall of the hemisphere between the olfactory bulb and the lamina terminalis. Here the sulcus in question separates the medial olfactory nucleus from the primordium hippocampi which occupies the roof. This sulcus I have called the *medial* sulcus limitans hippocampi, while the *lateral* sulcus limitans hippocampi separates the primordium hippocampi from the lateral olfactory nucleus in the lateral wall, (Johnston '11 a). In ganoids and teleosts nothing is evaginated but the olfactory bulb, there is no roof containing a primordium hippocampi and there is no medial olfactory nucleus in the medial wall

of an evaginated hemisphere. Therefore there is no medial sulcus limitans hippocampi.

Sheldon describes a nucleus commissuralis lateralis and a nucleus entopeduncularis which together seem to correspond to the writer's somatic nucleus. I see no reason for including the palaeostriatum, nucleus taeniae or the nucleus intermedius in the somatic area, as suggested by Sheldon. It is not clear what different writers mean by 'nucleus taeniae.' Sheldon's nucleus taeniae appears to be the same as Edinger's and Kappers', olfactory in function. Goldstein's nucleus taeniae is described as non-olfactory in function and it is probable that the body which he described belongs to the writer's somatic nucleus. Goldstein's nucleus entopeduncularis lies ventrally close to the chiasma and Sheldon can scarcely have identified it correctly in the carp. In Ameiurus there is a nucleus entopeduncularis independent of the somatic nucleus and ventral to it. This has apparently not been seen in the carp, unless it is included in the complex nucleus praeopticus. Sheldon's nucleus entopeduncularis is undoubtedly identical with the caudal part of my somatic nucleus.

The theoretical part of Dr. Sheldon's paper is a restatement of Herrick's theory regarding longitudinal columns in the telencephalon. The writer is unable to see that this theory has any relation to the facts set forth in the descriptive part of Sheldon's paper. The diagrams on plate 35 are evidently intended to illustrate this theory but not to give any facts for its support. Nothing is said in the paper to indicate the grounds on which the peculiar shifting about of the columns 1, 2 and 3 in these diagrams is based. If the writer's view is correct, the primordium hippocampi includes all or nearly all of the territory represented by the numerals *1*, *2* and *3* in figures 132, 133, 134. The somatic nucleus is not taken into account at all in these diagrams. In the text it is stated (p. 243), "the nucleus entopeduncularis [my somatic nucleus] probably belongs to the same column as the pars ventralis thalami, the pars ventro-lateralis of Herrick, which expands rostrally to form the palaeostriatum." That this view is untenable is clear from the simple fact that this nucleus is directly continuous caudally with the optic centers and closely related to

the other sensory centers which lie in Herrick's pars dorsalis thalami. The description of the palaeostriatum given by Sheldon shows that it has the same structure and connections as his lateral olfactory nucleus. I have specifically included the corresponding region in ganoids in my primordium hippocampi. It seems, then, that there is nothing in the telencephalon that may be regarded as the continuation of Herrick's column 3 of the diencephalon.

I have elsewhere ('11 b) criticized Herrick's schema because he sees the telencephalic continuation of the somatic sensory column of the diencephalon ('pars dorsalis thalami') in a structure of wholly different functional significance, namely, the lateral olfactory nucleus. Here in fishes the impropriety of this becomes evident from the fact that the pars dorsalis thalami has an actual structural continuation in the form of the somatic sensory nucleus of the telencephalon. Essentially the same condition prevails in all vertebrates.

Another difficulty with the application of Herrick's schema to the teleost brain is seen in the assignment of the substance adjacent to the taenia fornicis to column *2*, while the primordium hippocampi which is the telencephalic continuation of the most dorsal column *1* is placed in the lateral wall morphologically ventral to column *2*.

Herrick's schema when applied to the teleost brain appears artificial and inconsistent with the described facts.

Dr. Sheldon uses the term 'hemisphere' for the lateral lobes which are not evaginated. Professor Herrick ('10) has advocated the use of the term for the evaginated part of the telencephalon as distinguished from the telencephalon medium. The writer ('12 b) accepts this, and it is unfortunate that the same usage should not have been followed in all papers coming from Professor Herrick's laboratory.

It is thought that the comments in the foregoing paper will be clear to anyone who has before him the figures in the papers under discussion. The literature cited is also listed in those papers.

A MODEL DEMONSTRATING THE CHANGES IN POSITION AND PERITONEAL RELATIONS OF ABDOMINAL VISCERA DURING DEVELOPMENT

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THREE FIGURES

Differences in position of the abdominal viscera and in their peritoneal relations at various stages of development occupy the front rank of difficulties which confront the teacher of anatomy. Text-books are lavish in detail, and clearness of description is not lacking in either text or atlas. The fact remains, nevertheless, that but few students obtain a clear idea of the successive changes which occur in the abdominal cavity during growth from the simple arrangement of the gastro-intestinal canal and its common mesentery in the early foetus to the complicated conditions acquired at term.

Huntington meets the difficulty by comparing the dissection of the human abdomen with that of the cat, and a better illustration cannot be found to substantiate the value of comparative anatomy in the study of the development of human organs. Huntington's method consists in directing the student to make comparisons between the abdominal viscera of the cat and those of the human subject. Advantage is taken of the fact that the peritoneal arrangement of the adult cat is much like that of the human foetus in early development. By a series of manipulations of the viscera of the cat, those movements and displacements of the organs, which occur later in the foetus and which result in the permanent fixation of peritoneal relations, may be imitated.

Suggested by Huntington's method, a model is herewith presented which has been found to simplify to our students, the

explanation of processes by which the adult conditions in the human are attained. An apology for crudeness of the model should be offered. In its favor is the simplicity of its construction which is suggestive of simplicity rather than complexity in developmental processes. By manipulations of the organs represented, one may easily demonstrate the movements in rotation of

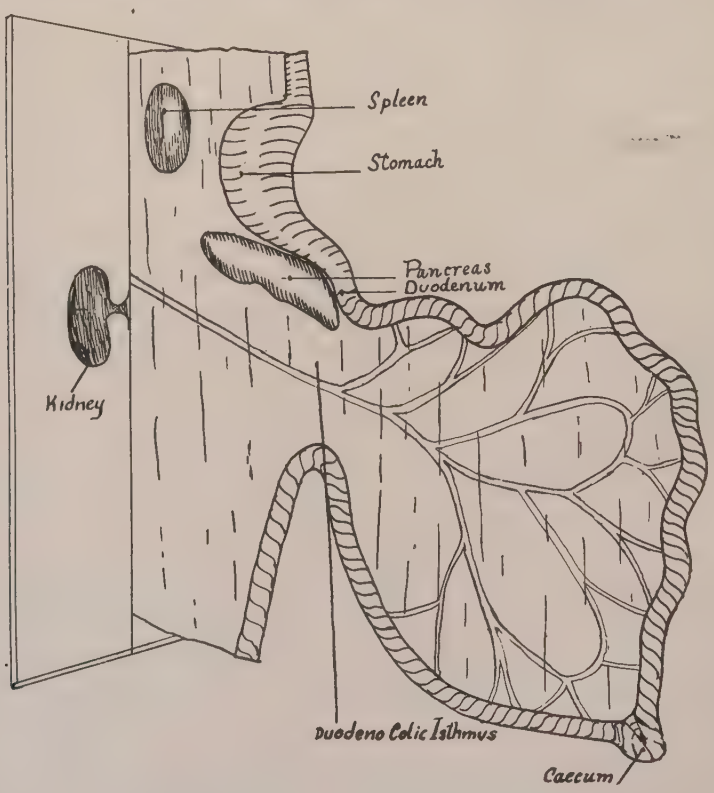

Fig. 1. Model representing gastro-intestinal canal before rotation of stomach and intestine.

the stomach and in torsion of the mesentery upon which the various changes in subsequent peritoneal relations essentially depend.

A board about 2 feet long and 7 inches wide (fig. 1) represents the posterior abdominal wall and a piece of muslin covering it represents the foetal parietal peritoneum. To the mid-line of the

board is attached a double layer of muslin, the common mesentery. In the upper part of this, the mesogastrium, are enclosed the stomach, pancreas and spleen, and its lower part is made up to represent the mesentry proper, which encloses the primitive intestinal loop with some development of intestinal coils in the upper

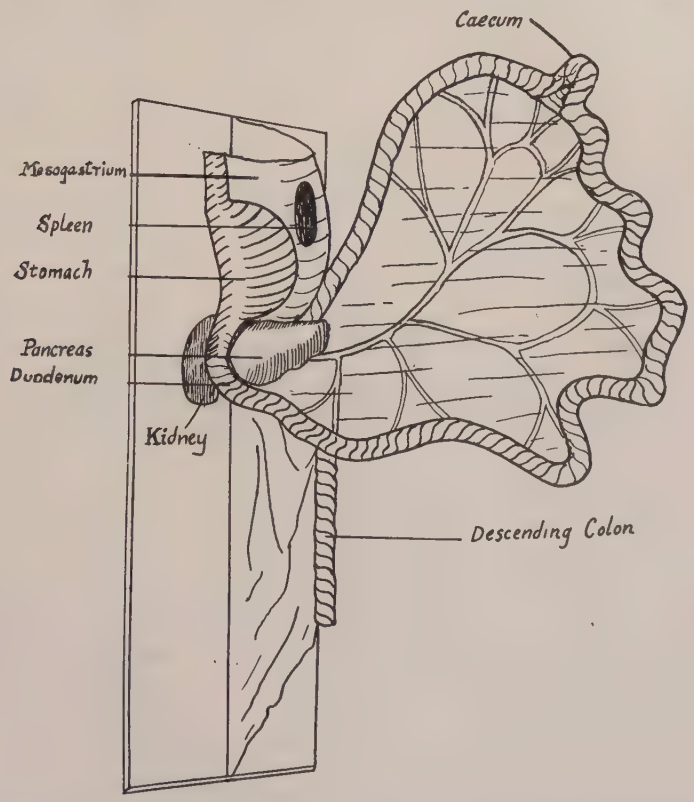

Fig. 2. Model showing change in relations of viscera attending rotation of stomach; figure shows also first step in twisting of the mesentery at the duodeno-colic isthmus.

limb of the loop and the caecum and the colon in the lower limb. The stomach, pancreas, spleen and kidneys are represented by cotton cushions and the intestinal canal by a soft cotton rope. The duodeno-colic isthmus is shown with the superior mesenteric vessels passing to the intestine.

The formation of the bursa omentalis and foramen of Winslow is illustrated by rotating the stomach from the sagittal to the transverse plane and by folding the mesogastrium (fig. 2). The ventral part of the fold then corresponds to the gastro-splenic omentum, the dorsal part of it to the posterior ligament of the spleen, and incidentally the displacement of the vagi nerves from the sides of the oesophagus to its ventral and dorsal surfaces is

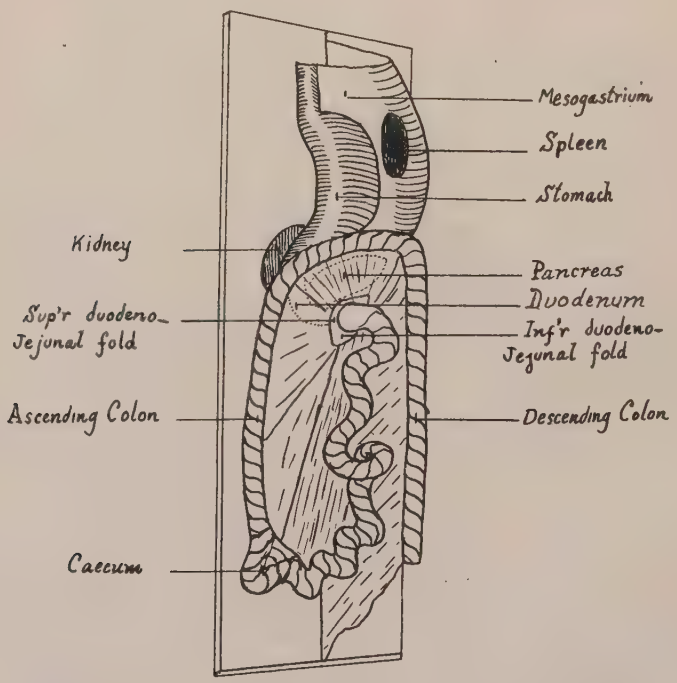

Fig. 3. Model shows position of abdominal viscera after rotation of stomach and complete twisting of mesentery.

demonstrated. Rotation of the stomach is accompanied by rotation of the pancreas and duodenum which latter are thus made to leave their sagittal position for the transverse. The movement of twisting of the mesentery is next performed: the small intestine is made to sweep from right to left below the large intestine, the caecum is carried up from left to right and above the small intes-

tine as in figure 2, and in its travel, the ascending meso-colon is made to pass over the duodenum and pancreas which have rotated with the stomach and have been made to lie in the transverse plane. After this twisting of the mesentery on the duodeno-colic isthmus has been understood, it is quite easy to explain how the ascending meso-colon, pressed backwards by the developing convolutions of the small intestine, eventually becomes a parietal layer, and how the pancreas and duodenum, which have been caught behind the ascending meso-colon, lose their embryonic peritoneal covering and appear to have developed in a post-peritoneal position (fig. 3). The jejunum is now seen emerging from behind the newly acquired parietal peritoneum and at the duodeno-jejunal angle, the superior and inferior duodeno-jejunal folds and fossae (fig. 3) may be imitated by the twisting of the mesentery.

The peritoneal and non-peritoneal areas of the kidneys are made clear and the occasional persistence of the descending meso-colon, in contrast to the more constant disappearance of the ascending meso-colon, is explained by the more extensive travel of the caecum and ascending colon, in comparison with the relatively stationary position of the descending colon.

Finally after the disappearance of the ascending meso-colon has been demonstrated, the new line of attachment of the root of the mesentery proper explains itself.

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

BRAIN AND SPINAL CORD, A manual for the study of the morphology and fibre-tracts of the central nervous system, Dr. Med. Emil Villiger, privatdozent in neurology and neuropathology in the University of Basel, translated by George A. Piersol, professor of anatomy in the University of Pennsylvania, from the third German edition, with 232 illustrations (43 in colors), 290 pages including index and bibliography, 1912, $4.00. J. B. Lippincott Company, Philadelphia and London.

This is an excellent translation of the German text (reviewed in The Anatomical Record, volume 5, number 4, pages 195 and 196, 1911) making this useful manual accessible to American students, to whom it is heartily recommended.

A CLINICAL MANUAL OF THE MALFORMATIONS AND CONGENITAL DISEASES OF THE FŒTUS, Professor Dr. R. Birnbaum, chief physician to the university clinic for women at Göttingen, translated and annotated by G. Blacker, M.D., 58 illustrations and 8 plates, 380 pages, 1912. P. Blakiston's Son and Company, Philadelphia.

MANUAL OF HUMAN EMBRYOLOGY, written by Charles R. Bardeen, Madison, Wis.; Herbert M. Evans, Baltimore, Md.; Walter Felix, Zurich; Otto Grosser, Prague; Franz Keibel, Freiburg, i.Br.; Frederic T. Lewis, Boston, Mass.; Warren H. Lewis, Baltimore Md.; J. Playfair McMurrich, Toronto; Franklin P. Mall, Baltimore, Md.; Charles S. Minot, Boston, Mass.; Felix Pinkus, Berlin; Florence R. Sabin, Baltimore, Md.; George L. Streeter, Ann Arbor, Mich.; Julius Tandler, Vienna; Emil Zuckerkandl, Vienna; edited by Franz Keibel, Professor in the University of Freiburg, i.Br. and Franklin P. Mall, Professor of Anatomy in the Johns Hopkins University, Baltimore, U.S.A., in two volumes, volume 2, with 658 illustrations, many of them in colors, 1032 pages including index, 1912. Volume 1 was published in 1911. The volumes are not sold separately, $20.00 for the set. J. B. Lippincott Company, Philadelphia and London.

ON MAKING SERIAL CELLOIDIN SECTIONS AND A STAIN FOR THE INTERCALATED DISCS OF CARDIAC MUSCLE

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I. A METHOD FOR MAKING SERIAL SECTIONS IN CELLOIDIN

Although several methods are now in use for making serial sections from celloidin blocks, all of them are more or less slow and involve some unnecessary manipulation. The method herein given is a modification of the celloidin sheet method for paraffin sections suggested by Huber[1] and of the Weigert method for making serial sections in celloidin.[2] It has given satisfactory results in my hands. In detail the method is as follows:

Select plates of glass of suitable size, say 5 by 7 inches, and thoroughly cleanse them. Coat one surface of the glass with the following solution and allow it to dry:

Saccharose-dextrin solution

Saccharose	3 grams
Dextrin	3 grams
Distilled water	100 cc.

Add a crystal of thymol to this solution to prevent fermentation and the growth of fungi.

After the above solution has thoroughly dried on the plate, it is then coated with a 4 per cent solution of celluloid in acetone. I have used coatings made with celloidin but do not find them as satisfactory for the following reasons:

(1) Celloidin sheets are more fragile and thus more easily torn. (2) Celloidin sheets stain by many of the dyes, whereas

[1] Huber, G. Carl, Laboratory work in histology, p. 31, 1900.

[2] Wiegert, C., Zeitschr. für Wiss. Mikros., Bd. 2, 1885, p. 490.

celluloid does not. (3) Celloidin sheets are not as transparent as celluloid sheets. (4) These celluloid sheets may be made up and kept on hand for future use, dried if desired, while celloidin sheets cannot, for the celloidin will crack upon drying.

When the sections are cut, they should be placed on the celluloid sheets in the desired order and should be moistened from time to time to keep them from drying out. After the plate is filled with sections, it should be blotted with a smooth surfaced toilet paper to remove the excess of alcohol. The surface should now be sprayed with a 1 or 2 per cent solution of celloidin in equal parts of ether and absolute alcohol, to cause the sections to adhere to the celluloid sheet. The spraying is best done by the aid of an atomizer. Allow this to dry partially, then immerse the plate containing the sheet in 70 per cent alcohol and then in water. The water will dissolve the saccharose-dextrin solution and the celluloid sheet containing the sections will then float off. The sheet may now be preserved in 75 or 80 per cent alcohol and stained at some future time, or, it may be passed through the staining reagents at once and finally cleared in any clearing fluid suitable for celloidin sections. The sections are now separated into strips of the desired length by means of a roller-paper cutter, or cut apart with a sharp knife, and mounted in their serial order on slides properly numbered so as to keep the sections in continuous series.

II. ON STAINING THE INTERCALATED DISCS IN CARDIAC MUSCLE

J. L. Bremer recommended[3] Mallory's phosphotungstic acid hematoxylin for the staining of intercalated discs in heart muscle. I find that by Bremer's method the striations and intercalated discs stain well, but to get a good nuclear stain it is necessary to stain longer and by so doing the preparation is made too dark. With the method given below I have had good results.

1. Fix in Zenker's fluid or saturated aqueous mercuric bichloride. Formalin will do but the results are not so good as after either of the above fixing agents.

[3] Anat. Rec., July, 1910.

2. Wash, dehydrate, and embed in either celloidin or in paraffin.

3. Cut thin sections and bring them down to distilled water.

4. Deeply overstain (6 to 24 hours at room temperature) in a well ripened solution of either Mallory's phosphotungstic acid hematoxylin or phosphomolybdic acid hematoxylin. The same results may be more quickly obtained by placing in the paraffin oven at 45° or 50°C. for from forty-five minutes to two hours.

5. Wash in water and place in a 0.25 per cent solution of potassium permanganate until the blue black stain begins to turn brown. This time varies from two to fifteen minutes, depending on the length of time the sections remain in the hematoxylin.

6. Wash hastily in distilled water to remove excess of potassium permanganate and transfer to a 1 per cent aqueous solution of acid potassium sulphite until the preparation assumes a steel-gray cast. If the preparation now be cleared and looked at under the microscope, it will be found that only the intercalated discs are stained. I have found that the desired decolorization is more readily accomplished and have obtained a sharper and deeper differentiation of the discs in sections stained with phosphomolybdic acid hematoxylin than with phosphotungstic acid hematoxylin.

7. Wash the sections with water and place in Mayer's acid hematein for five minutes, to stain the nuclei. I add hematoxylin to Mayer's formula instead of hematein. I like it better because it stains the nuclei more deeply.

8. Wash well in tap water or in distilled water to which a trace of ammonium carbonate or hydroxide has been added.

9. Place in a 1 per cent aqueous solution of eosin or erythrosin for ten or fifteen minutes.

10. Wash in water, dehydrate, clear, and mount in balsam.

In well stained preparations, the nuclei are stained deeply blue, the muscle fibrillae red and the intercalated discs stand out sharply as a deep blue black.

The formulae for the stains used are as follows:

Mallory's phosphotungstic acid hematoxylin

Hematoxylin	0.1	gram
Distilled water	80	cc.
10 per cent aqueous phosphotungstic acid	20	cc.
Peroxide of hydrogen (U. S. Ph.)	0.2	cc.

Mallory's phosphomolybdic acid hematoxylin

0.5 per cent aqueous phosphomolybdic acid	200	cc.
Hematoxylin	1.75	gram

Mayer's acid hemalum

Hematein (or hematoxylin)	1	gram
90 per cent alcohol	50	cc.
Alum	50	grams
Water	1000	cc.
Acetic acid (glacial)	50	cc.

ON THE RELATIVE GROWTH OF THE COMPONENT PARTS (HEAD, TRUNK AND EXTREMITIES) AND SYSTEMS (SKIN, SKELETON, MUSCULATURE AND VISCERA) OF THE ALBINO RAT

C. M. JACKSON AND L. G. LOWREY

From the Anatomical Laboratory of the University of Missouri

TWO FIGURES

To comprehend fully the growth of the body, the following data are required: (1) the growth of the body as a whole; then an analysis to determine (2) the growth of the principal parts; (3) the growth of the various systems; (4) the growth of the individual organs; and finally (5) the growth of the ultimate constituent tissues and cells. Observations upon these various phases of growth in different animals are scattered through the literature, but in no case are they sufficiently complete to afford a comprehensive view of the process of growth in any individual species. In the case of the albino rat, the growth of the body as a whole and of the central nervous system has been carefully studied by Donaldson and his associates. The present paper will give a partial analysis of the growth process in this animal, including the relative growth from birth to maturity in the various constituent parts and systems of the body. A more extensive study of the growth and variation in the individual viscera will be published soon in a separate paper.

MATERIAL AND METHODS

Ninety-three albino or white rats (Mus norvegicus albinus) were utilized for the present paper. These include 18 newborn (9 males, 9 females); 19 at 1 week (8 m., 11 f.); 13 at 3 weeks (7 m., 6 f.); 14 at 6 weeks (6 m., 8 f.); 10 at 10 weeks (5 m., 5 f.); 13 at about 5 months (6 m, 7 f.); and 6 at about 1 year (4 m., 2 f.). They

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were fed daily with wheat bread soaked in whole milk, and a supply of chopped corn was kept constantly in the cages. In addition, they were fed fresh meat (beef) once a week. They were well cared for in an animal house, and represent well-nourished, healthy animals. In a few of the older animals, the lungs were infected, but none is included in which the infection was apparently sufficient to affect the general nutrition or vigor.

The various litters were kept separate and are indicated in the tables. While the number of animals is not large (on account of the laborious method of dissection), it is sufficient to give some idea of the average condition and of the extent of variation. Since the personal equation is likely to enter to a certain extent in the process of separating the muscular and skeletal systems, etc., in dissection, those litters dissected by Lowrey are designated by the prefix A or M with the litter number. Those without prefix (including all the observations given for the head, trunk and extremities) were dissected by Jackson.

The method of dissection was as follows. The animal was taken in the morning before feeding and killed by chloroform. The gross body weight, and the lengths of body and tail were recorded. The head was then removed (just posterior to the foramen magnum and anterior to the larynx) and weighed. In the meantime, the trunk was suspended and the blood (unmeasured) was allowed to escape. Then the viscera were carefully removed and weighed individually (including brain, spinal cord, eyeballs, thyroid, thymus, heart, lungs, liver, spleen, stomach and intestines, both with contents and empty, suprarenals, kidneys and gonads. Urine was estimated if present. The extremities were separated at the shoulder and hip joints and weighed. The skin was next removed (including ears, claws and adherent subcutaneous tissue) and weighed. Then the musculature with skeleton was weighed, the few remaining additional structures (genitalia, large vessels, pharynx and oesophagus, larynx and trachea, and masses of fat connected with the musculature) having been carefully removed. Finally the musculature was carefully dissected off and the skeleton, including bones, cartilages and ligaments, was weighed. This weight, subtracted from that

of the skeleton and musculature together, gives the weight of the musculature, including the tendons. Evaporation was reduced to a minimum by keeping the various structures in a closed moist container, so far as possible. The net body weight, which is the gross body weight minus contents of stomach, intestines and urinary bladder, was used as the basis in calculating the percentage weights. The percentages are therefore slightly higher than they would be if calculated upon the gross body weight. The difference is not of material importance in the case of the albino rat, however, as the contents do not average more than 5 per cent of the body at the ages observed (excepting at 6 weeks, where the average was about 8 per cent.

The observations were grouped at seven ages, chosen for the following reasons. At 1 week the weight at birth has about doubled. At 3 weeks, it has about doubled again, and this moreover is the age at which the animal is usually weaned. At 6 weeks, the body weight has again about doubled, and the animal is well established upon its permanent diet. Ten weeks represents the age of puberty, and the body weight of 6 weeks has again about doubled. At 1 year, the body weight has again nearly doubled, and this represents nearly the adult weight. Five months was arbitrarily selected as the time when the body weight is approximately half way between those of 10 weeks and 1 year. While therefore observations are not available for the various intermediate age periods, these are sufficiently close together so that no important change in the relative weights of the constituent parts is likely to be overlooked. Moreover, on account of the variations at the different ages in the body weights, these form a fairly continuous series; and the relative weights of the various constituent parts are apparently more closely correlated with the body weight than with the age.

Observations (by Jackson) upon 5 wild gray or brown rats (Mus norvegicus) are also included for comparison, in tables 5 and 6. These rats were captured by traps in barns, and were probably chiefly grain-fed.

For the sake of economy of space, and since the present paper is concerned primarily with the relative weights, the percentage

weights only are recorded in the tables. The absolute weight of the body (net) is given in all cases, however, from which the absolute weight of the individual parts can easily be calculated if desired. Moreover, the original data will be deposited in The Wistar Institute of Anatomy in Philadelphia, where they will be accessible to any who may care to use them.

RELATIVE GROWTH OF THE COMPONENT PARTS

1. Head (tables 1, 2, 5; fig. 1). In the limited series of data given in table 1, it will be noted that, on the average (table 2), the head increases from 21.65 per cent of the body in the newborn to 23.70 per cent at 1 week; decreasing to 20.22 per cent at 3 weeks, 11.80 per cent at 6 weeks, 9.56 per cent at 10 weeks, 9.42 per cent at 5 months, and 9.29 per cent at 1 year. In a much larger series, however, including 20 or more of each sex at each age (observed by Jackson in a study of the growth of organs), the relative weights were found to average somewhat higher, being 23.43 per cent, 25.74 per cent, 24.27 per cent, 15.17 per cent, 11.21 per cent, 10.47 per cent and 10.75 per cent, respectively (data in parentheses in table 2.) This is to be explained partly on account of individual variations in the smaller series, and partly as due to correlation with the body weight, which in all cases averages heavier in the smaller than in the larger series of corresponding age. In constructing the diagram in Fig. 1, the data from the larger series were used.

No difference in the relative weight of the head (aside from that due to different body weight) is evident between the sexes; and the small series of observations on the gray rat (table 5) shows the head to be of approximately the same relative size as in the albino.

Fig. 1 Change in the percentage weight of the component parts of the albino rat. The width in the vertical direction) of each strip is proportional to the percentage weight of the corresponding part. The percentage weight is indicated for every part at each of the ages. Up to the age of 10 weeks (that is, in the ruled portion of the figure), the horizontal distance is drawn to scale, proportional to the age. Beyond 10 weeks, the horizontal distance is not in proportion to the age.

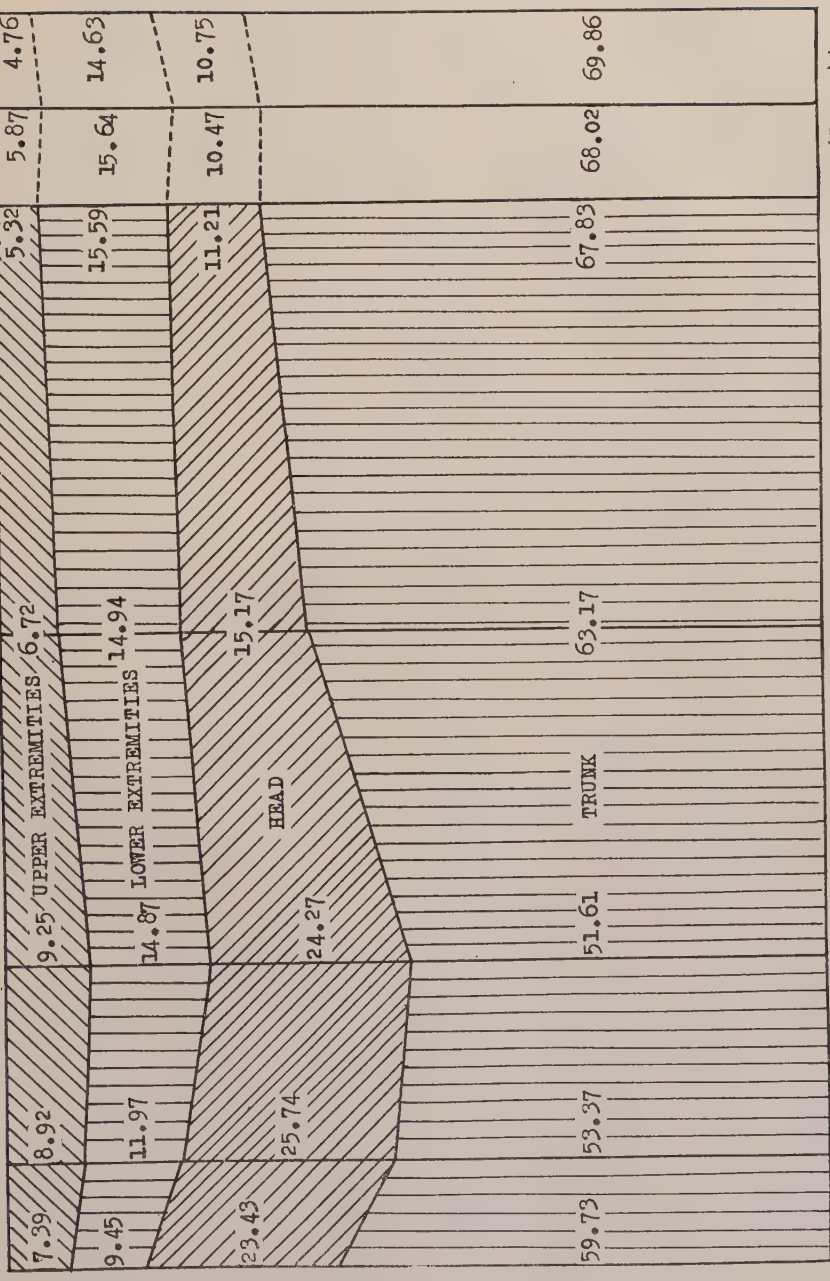

UPPER EXTREMITIES
LOWER EXTREMITIES
HEAD
TRUNK
7.39
8.92
9.25
6.72
9.45
11.97
14.87
14.94
23.43
25.74
24.27
15.17
59.73
53.37
51.61
63.17
5.32
5.87
4.76
15.59
15.64
14.63
11.21
10.47
10.75
67.83
68.02
69.86
Newb. 1 wk.
3 wk.
6 wk.
10 wk.
(5 mo.)(1 yr.)

TABLE 1

Albino rat—Percentage weight of the head, trunk and extremities

Newborn

LITTER NUMBER	SEX	NET BODY WEIGHT	HEAD	UPPER EXTREMITY	LOWER EXTREMITY	TRUNK
		grams	*per cent*	*per cent*	*per cent*	*per cent*
47	m.	5.88	22.60	7.27	9.70	60.43
47	m.	5.96	22.40	7.57	8.49	61.54
47	m.	6.25	21.12	8.45	8.00	62.43
62	f.	4.27	21.08	7.07	9.62	62.23
58	f.	5.09	21.59	7.27	10.41	60.73
63	f.	4 78	21.13	6.69	10.46	61.72

One week

49	m.	11.96	23.06	7.27	12.88	56.79
46	m.	14.95	21.92	9.23	12.44	56.41
59	m.	9.55	25.03	8.90	11.62	54.45
60	f.	9.63	24.68	8.00	11.22	56.10
49	f.	10.33	23.54	9.37	11.13	55.96
46	f.	13.23	23.98	10.74	12.55	52.73

Three weeks

53b	m.	25.80	19.49	7.40	15.20	57.91
53b	m.	29.08	18.97	10.94	15.37	54.72
53b	m.	29.89	18.24	9.50	14.27	57.99
57	f.	17.34	24.16	9.17	14.65	52.02

Six weeks

50a	m.	82.50	11.25	6.19	14.63	67.93
50a	f.	76.80	12.15	5.90	14.88	67.07
50a	f.	78.60	11.82	8.22	14.90	65.06
50a	f.	78.80	11.97	6.57	15.37	66.09

Ten weeks

50b	m.	175.20	8.56	5.21	15.77	70.46
50b	f.	120.30	9.98	5.24	16.45	68.33
50b	f.	130.20	10.14	5.50	14.54	69.82

TABLE 1—Continued

Five months[1]

LITTER NUMBER	SEX	NET BODY WEIGHT	HEAD	UPPER EXTREMITY	LOWER EXTREMITY	TRUNK
		grams	*per cent*	*per cent*	*per cent*	*per cent*
28	m.	239.40	8.35	5.43	14.91	71.31
28	f.	171.50	10.00	6.01	15.80	68.19
37	f.	161.30	9.92	6.17	16.20	67.71
			One year			
39	m.	229.20	9.10	4.93	14.62	71.35
37	m.	276.40	9.18	4.67	14.54	71.61
39	f.	161.10	9.60	4.68	14.73	70.99

[1] The third individual in this group was 8 months old.

TABLE 2

Albino rat—Average percentage weight of head, trunk and extremities at various ages (from table 1)

AGE	HEAD	UPPER EXTREMITY	LOWER EXTREMITY	TRUNK
	per cent	*per cent*	*per cent*	*per cent*
Newborn.....	21.65 (23.43)[1]	7.39	9.45	61.51 (59.73)[1]
One week.....	23.70 (25.74)	8.92	11.97	55.41 (53.37)
Three weeks.....	20.22 (24.27)	9.25	14.87	55.66 (51.61)
Six weeks.....	11.80 (15.17)	6.72	14.94	66.54 (63.17)
Ten weeks.....	9.56 (11.21)	5.32	15.59	69.53 (67.83)
Five months.....	9.42 (10.47)	5.87	15.64	69.07 (68.02)
One year.....	9.29 (10.75)	4.76	14.63	71.32 (69.86)

[1] Figures in parentheses indicate the average percentages of the head (and corresponding percentages of the trunk) in a much larger series, including 20 or more of each sex at each age.

It is thus a remarkable fact that for a short time after birth the head of the albino rat grows more rapidly than the remainder of the body, probably reaching its maximum relative size in the second week. It is well known that the head of animals in general is relatively largest during early embryonic life, and that it declines during the later prenatal period (cf. Jackson). After birth, since it is still relatively large as compared with the adult, the head would naturally be expected to continue to decline in

TABLE 3

Albino rat—Percentage weight of skin, skeleton, musculature and viscera

LITTER NUMBER	SEX	NET BODY WEIGHT	SKIN	SKELETON	MUSCULATURE	VISCERA	REMAINDER
		grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Newborn							
47	m.	5.88	18.30	13.95	21.26	17.38	29.11
47	m.	5.96	16.90	15.61	22.56	19.00	25.93
47	m.	6.25	20.29	15.84	24.66	17.78	21.43
A22	m.	4.87	21.50	19.70	23.40	15.94	19.46
A23	m.	5.22	19.60	19.50	18.90	17.50	24.50
A24	m.	4.57	22.30	23.50	20.60	18.50	15.10
A34	m.	4.20	18.15	13.70	26.70	18.13	23.32
A34	m.	4.53	18.00	14.70	26.35	16.68	24.27
A34	m.	4.54	21.24	16.59	25.25	17.10	19.82
62	f.	4.27	19.01	16.43	25.00	18.48	21.08
58	f.	5.09	20.43	14.33	26.54	18.21	20.49
63	f.	4.78	19.46	16.50	27.85	19.68	16.51
A25	f.	4.44	21.00	20.40	21.30	20.17	17.13
A29	f.	3.58	21.20	24.80	23.90	19.86	10.24
A34	f.	4.47	19.80	14.80	29.80	17.13	18.47
A34	f.	4.17	17.65	15.74	24.42	18.17	24.02
A34	f.	3.38	20.25	18.00	24.62	17.15	19.98
A34	f.	4.26	20.37	16.80	25.60	18.02	19.21
One week							
46	m.	14.95	29.36	15.78	22.41	17.43	15.02
49	m.	11.96	31.52	17.06	24.58	15.29	11.55
59	m.	9.55	25.23	17.49	24.60	19.68	13.00
A22	m.	10.30	29.90	22.20	22.50	19.92	5.48
A32	m.	8.99	23.96	17.70	22.53	18.61	17.20
A32	m.	10.76	25.45	18.90	19.25	20.13	16.27
M9	m.	8.11	24.10	19.40	23.70	20.98	11.82
M9	m.	9.12	23.50	18.60	23.50	20.42	13.98
46	f.	13.23	29.37	14.36	24.35	18.26	13.66
49	f.	10.33	31.75	15.30	26.52	15.55	10.88
60	f.	9.63	25.34	17.54	24.00	18.55	14.87
A21	f.	8.85	28.60	23.80	23.40	18.41	5.79
A24	f.	7.65	24.20	23.60	19.00	20.63	12.57
A28	f.	6.98	23.30	22.20	22.40	20.50	11.60
A32	f.	8.76	21.30	16.10	19.60	19.80	23.20
A32	f.	10.63	27.00	18.05	21.63	19.16	14.16
A33	f.	8.07	21.78	18.52	22.47	20.09	17.14
A33	f.	7.43	22.05	17.60	23.30	20.37	16.68
A33	f.	9.95	23.96	16.70	23.90	20.46	14.98

TABLE 3—Continued

LITTER NUMBER	SEX	NET BODY WEIGHT	SKIN	SKELETON	MUSCULATURE	VISCERA	REMAINDER
		grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Three weeks							
53b	m.	25.80	29.20	14.90	29.80	22.08	4.02
53b	m.	29.08	26.39	16.72	28.08	21.59	7.22
53b	m.	29.89	27.49	13.11	27.69	20.38	11.33
A32	m.	25.91	18.70	17.30	27.10	20.79	16.11
M9	m.	25.78	22.10	15.70	26.70	20.61	14.89
M9	m.	25.40	21.50	15.60	26.30	20.24	16.36
M9	m.	26.50	19.50	15.80	28.30	22.35	14.05
57	f.	17.34	22.26	19.49	30.22	24.82	3.21
A26	f.	18.41	23.55	21.08	20.14	20.57	14.66
A32	f.	23.72	21.10	16.65	25.66	21.90	14.69
A32	f.	25.70	19.80	17.10	27.50	20.91	14.69
A33	f.	24.40	18.80	15.90	25.30	20.09	19.91
A33	f.	24.30	20.55	16.43	26.77	20.35	15.90
Six weeks							
50a	m.	82.5	25.86	11.67	35.13	20.80	6.54
A24	m.	54.1	20.00	15.15	29.60	20.37	14.88
A24	m.	41.5	20.20	20.10	26.10	21.79	11.81
A28	m.	56.6	19.80	14.70	33.70	20.78	11.02
A29	m.	62.9	17.90	14.16	34.90	21.47	11.57
A29	m.	62.9	17.63	14.31	34.45	22.89	10.72
50a	f.	76.8	25.53	10.50	28.56	18.35	17.06
50a	f.	78.6	24.94	12.63	33.77	19.22	9.44
50a	f.	78.8	25.55	11.82	35.25	18.41	8.97
A29	f.	54.7	16.74	14.13	33.82	21.27	14.04
A29	f.	59.8	21.00	13.60	34.60	19.98	10.92
A29	f.	64.8	19.10	13.40	33.90	19.72	13.88
A31	f.	64.7	19.70	14.15	31.42	20.41	14.32
A31	f.	62.7	18.84	15.35	32.60	20.01	13.20
Ten weeks							
50b	m.	175.2	20.95	11.07	43.15	16.15	8.68
A28	m.	109.2	15.60	12.00	39.60	17.57	15.23
A28	m.	134.3	16.80	10.40	40.90	16.69	15.21
A30	m.	144.7	18.70	12.20	37.40	15.72	15.98
A30	m.	187.2	18.80	10.00	37.90	14.89	18.41

TABLE 3—Continued

Ten weeks—Continued

LITTER NUMBER	SEX	NET BODY WEIGHT	SKIN	SKELETON	MUSCULATURE	VISCERA	REMAINDER
		grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
50b	f.	120.3	22.28	12.39	49.13	14.97	1.23
50b	f.	130.2	21.34	12.90	41.78	15.75	8.23
A26	f.	109.9	17.93	11.70	41.42	16.32	12.63
A28	f.	108.4	16.70	12.20	42.30	14.99	13.81
A29	f.	122.2	17.80	11.90	37.80	17.22	15.28
			Five months[1]				
28	m.	239.4	22.76	13.24	41.39	15.39	7.22
A22	m.	192.3	18.80	12.30	42.10	12.99	13.81
A26	m.	203.4	18.50	10.40	43.90	13.26	13.94
A26	m.	232.4	18.90	10.00	43.10	13.42	14.58
A26	m.	195.4	18.30	10.20	41.20	14.02	16.28
A26	m.	249.4	18.00	9.37	44.30	13.13	15.20
37	f.	161.3	20.36	10.54	46.50	12.53	10.07
28	f.	171.5	17.79	14.58	46.25	14.53	6.85
A22	f.	157.1	18.80	11.60	40.20	16.19	13.21
A22	f.	158.0	16.20	12.20	39.70	15.46	16.44
A22	f.	158.5	15.70	10.90	41.90	17.24	14.26
A22	f.	128.2	14.50	11.80	41.90	18.70	13.10
A26	f.	149.0	17.20	12.90	41.90	15.21	12.79
			One year[1]				
39	m.	229.2	15.93	9.69	46.51	14.86	13.01
37	m.	276.4	20.62	11.61	42.65	13.63	11.49
A—	m.	253.5	18.45	10.45	46.33	11.49	13.28
A—	m.	281.6	13.75	7.35	50.50	12.17	16.23
39	f.	161.1	21.97	10.86	41.15	13.85	12.17
3	f.	206.0	16.99	15.53	45.46	13.82	8.20

[1] The first female of the 5 months' list was 8 months old. The age of the third and fourth males of the year list was not exactly known, but it was in the neighborhood of a year.

relative size, and we meet no data or statements to the contrary in the literature. Whether this early postnatal acceleration of the head growth is peculiar to the rat is therefore unknown, as well as its relations to prenatal growth.

It is interesting to note that the maximum relative weight of the head of the young rat (about 26 per cent) is nearly the same as that observed by Jackson for the human newborn; and that the adult rat head (9 to 10 per cent) is also not far from that of the human (6 to 10 per cent) as given by Meeh and Harless. Few data are available for comparison with other forms. Martiny, in 3 groups of beef cattle (10 in each group), finds the head forming an average of 2.7 per cent to 2.9 per cent of the body weight. Lawes and Gilbert give data showing the head in 2 fat calves to average 5.5 per cent of the body weight; and in 16 adult heifers and steers, 2.7 per cent. In 249 sheep they find the head averages 2.9 per cent of the body, varying from 3.6 per cent in 5 thin yearlings to 2.5 per cent in 45 very fat sheep, aged $1\frac{3}{4}$ years. Lowrey finds that in the pig the head decreases from about 22.3 per cent (late fetus) to an average of 6.3 per cent in the adult.

2. *Extremities* (tables 1, 2, 5; fig. 1). In spite of individual variations shown in table 1, the upper extremities on the average (table 2, fig. 1) are seen to increase from 7.39 per cent of the body at birth to 8.92 per cent at 1 week, and to 9.25 per cent at 3 weeks. From this maximum relative size, they decrease rapidly to 6.72 per cent at 6 weeks, and thereafter more slowly to an average of 4.76 per cent at 1 year.

The lower extremities show a continuous relative increase, which is at first more rapid, from an average of 9.45 per cent at birth to 11.97 per cent at 1 week, and to 14.87 per cent at 3 weeks. Thereafter the increase is slower, reaching a maximum of 15.64 per cent at 5 months, with an apparent later slight decrease to 14.63 per cent at 1 year.

The number of observations is insufficient to show any difference between the sexes as to relative weight of the extremities, if such exists. Similarly, the data on the gray rat (table 5) reveal no significant difference from the albino.

TABLE 4

Albino rat—Average percentage weights of skin, skeleton, musculature, viscera and remainder (from table 3)

AGE	NUMBER AND SEX	SKIN	SKELETON	MUSCULATURE	VISCERA	REMAINDER
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Newborn	18 (9m. 9f.)	19.75 (m.–19.59 f. –19.91)	17.27 (m.–17.01 f. –17.53)	24.37 (m.–23.30 f. –25.45)	18.05 (m.–17.56 f. –18.54)	20.56 (m.–22.55 f. –18.57)
One week	19 (8m. 11f.)	25.88 (m.–26.63 f. –25.33)	18.47 (m.–18.39 f. –18.52)	22.82 (m.–22.88 f. –22.78)	19.17 (m.–19.06 f. –19.25)	13.68 (m.–13.04 f. –14.14)
Three weeks	13 (7m. 6f.)	22.38 (m.–23.55 f. –21.01)	16.60 (m.–15.59 f. –17.78)	26.89 (m.–27.71 f. –25.93)	21.28 (m.–21.15 f. –21.44)	12.85 (m.–12.00 f. –13.84)
Six weeks	14 (6m. 8f)	20.91 (m.–20.23 f. –21.42)	13.98 (m.–15.02 f. –13.20)	32.70 (m.–32.31 f. –32.99)	20.39 (m.–21.35 f. –19.67)	12.03 (m.–11.09 f. –12.73)
Ten weeks	10 (5m. 5f.)	18.69 (m.–18.17 f. –19.21)	11.67 (m.–11.13 f. –12.22)	41.14 (m.–39.79 f. –42.49)	16.03 (m.–16.20 f. –15.85)	12.47 (m.–14.70 f. –10.23)
Five months	13 (6m. 7f.)	18.14 (m.–19.21 f. –17.22)	11.54 (m.–10.92 f. –12.07)	42.64 (m.–42.66 f. –42.62)	14.77 (m.–13.70 f. –15.69)	12.91 (m.–13.51 f. –12.39)
One year	6 (4m. 2f.)	17.95 (m.–17.19 f –19.48)	10.91 (m.– 9.78 f. –13.19)	45.43 (m.–46.50 f. –43.30)	13.30 (m.–13.04 f. –13.84	12.40 (m.–13.50 f. –10.19)

Scarcely any data are found in the literature as a basis for comparison with other forms. The observations made upon domestic animals at the various agricultural experiment stations usually follow the 'butcher's cuts,' which unfortunately do not correspond to the anatomical subdivisions. The only data available are the observations by Jackson, Meeh and Harless (l. c.), which indicate that in the human newborn the upper extremities form about 10 per cent of the body, which is about the same as in the adult; and that the lower extremities form about 20 per cent in the newborn and about 35 per cent in the adult. Thus, as might be expected, the extremities, especially the lower, are relatively much larger in man than in the rat. No observations are recorded for stages between the newborn and the adult.

TABLE 5

Gray (brown) rat—Percentage weight of head, trunk and extremities

SEX	NET BODY WEIGHT	HEAD	UPPER EXTREMITY	LOWER EXTREMITY	TRUNK
	grams	*per cent*	*per cent*	*per cent*	*per cent*
m.	65.0	14.66	5.95	13.88	65.51
m.	95.4	12.17	5.83	15.34	66.66
f.	107.5	10.18	5.58	15.81	68.43
m.	164.0	9.27	5.24	14.94	70.55
f.	254.0[1]	7.85	5.02	13.68	73.45

[1] Including gravid uterus, which weighed 13.76 grams.

3. *Trunk* (tables 1, 2, 5; fig. 1). The trunk weight was estimated by subtracting the weight of the head and extremities from the net body weight. The loss of blood (which was usually comparatively small) therefore falls entirely to the trunk. In the parentheses in table 2 are given the figures for the trunk corresponding to the larger series, as explained for the head, and these are also utilized for the diagram in figure 1. It is evident that both sets agree in showing that the trunk decreases notably in relative size from about 60 per cent of the body at birth to a minimum (52 to 55 per cent) at the first to third weeks (corresponding to the relative increase of the head and extremities), and thereafter increases steadily, reaching its maximum relative size (about 70 per cent) in the oldest and largest animals observed.

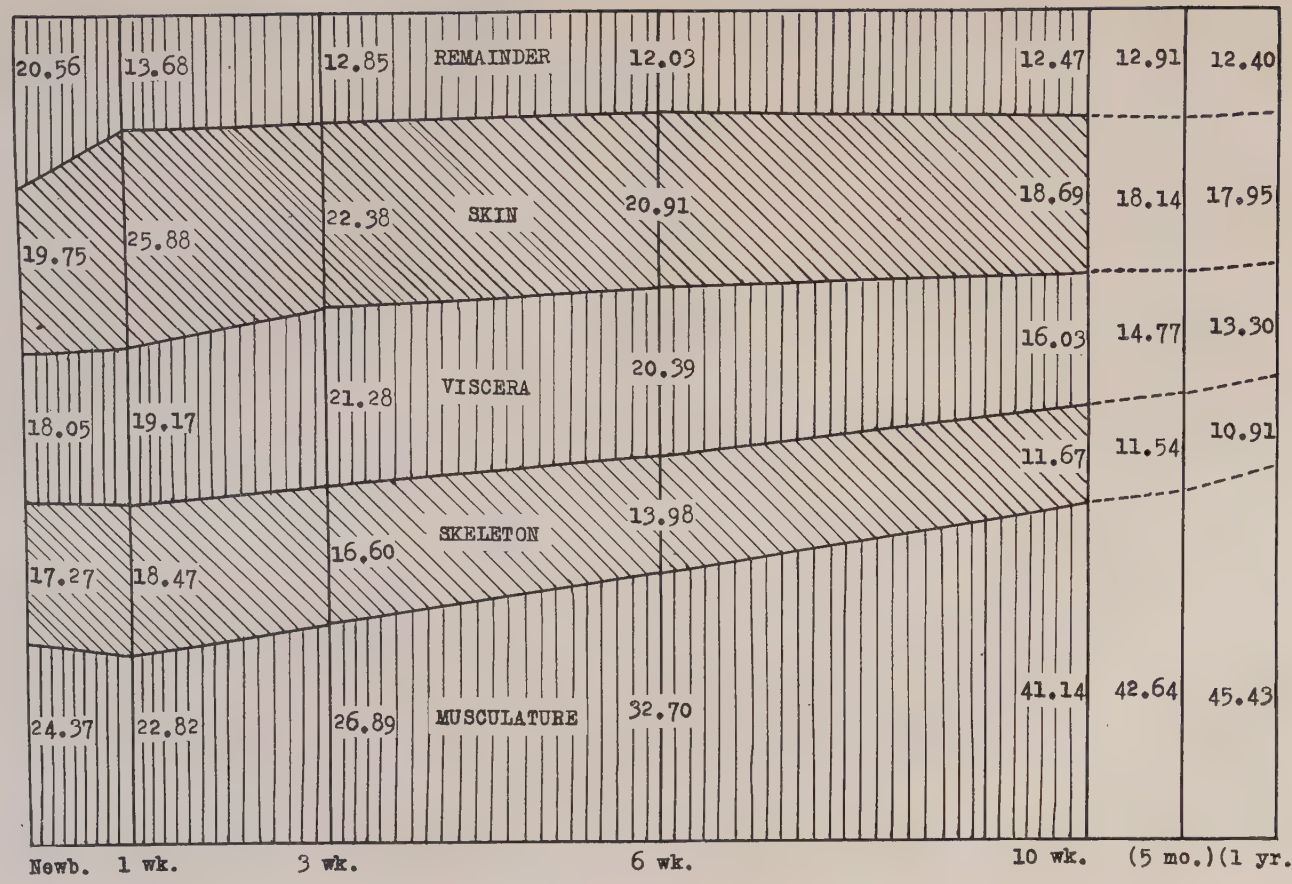
No differences are evident on comparing the sexes, or the data for the gray rat (table 5). The only data found in the literature for comparison with other forms are again those of Jackson and Meeh (l. c.), which show that the trunk forms about 45 per cent of the body in the human newborn, and about 48 per cent in the adult. Thus the human trunk is relatively smaller, corresponding to the relatively larger extremities.

As Jackson has pointed out for the human body, it may likewise be noted in the rat that the intensity of growth seems to pass over the body somewhat like a wave, reaching its maximum first in the head and upper extremity, and later passing backward along the trunk to the abdominal portion and lower extremity. This relation is evident in figure 1. It is furthermore evident from this figure that the adult relations of the component parts of the body have practically been reached at 10 weeks. The only changes apparent thereafter are a very slight relative increase in the trunk, compensated by a corresponding decrease in the head and extremities.

RELATIVE GROWTH OF THE VARIOUS SYSTEMS

1. Skin (tables 3, 4, 6; fig, 2). As shown by table 4 and in figure 2, the integument (including claws and adherent subcutaneous tissue) grows with remarkable rapidity during the first week, the average increasing from 19.75 per cent to 25.88 per cent of the whole body. The numbers at each age are too large and the uniformity too great to account for this on the score of possible individual variations. This unquestionable increase is however somewhat difficult to explain. It is not due to the development of the hair coat, for this does not become well developed until the second and third weeks. Neither is it apparently due to any unusual accumulation of fat in the subcutaneous tissue.

Fig. 2 Change in the percentage weight of the various systems of the albino rat. The width (in the vertical direction) of each strip is proportional to the percentage weight of the corresponding system. The percentage weight is indicated for every system at each of the ages. Up to the age of 10 weeks (that is, in the ruled portion of the figure), the horizontal distance is drawn to scale, proportional to the age. Beyond 10 weeks, the horizontal distance is not in proportion to the age.


20.56
13.68
12.85
REMAINDER
12.03
12.47
12.91
12.40
19.75
25.88
22.38
SKIN
20.91
18.69
18.14
17.95
18.05
19.17
21.28
VISCERA
20.39
16.03
14.77
13.30
17.27
18.47
16.60
SKELETON
13.98
11.67
11.54
10.91
24.37
22.82
26.89
MUSCULATURE
32.70
41.14
42.64
45.43
Newb.
1 wk.
3 wk.
6 wk.
10 wk.
(5 mo.)
(1 yr.)

After the first week the skin decreases in relative weight, at first more rapidly, and later more slowly, reaching an average of a little less than 18 per cent at 1 year. There are no noteworthy differences apparent between the sexes, or in the gray rat (table 6).

Numerous observations upon the integument in other forms are available for comparison, though chiefly for the adult. According to Vierordt, the average for the human newborn is 19.7 per cent; for the adult, 17.8 per cent. Welcker and Brandt record observations upon a large number of species, including besides mammals many birds (14 to 28 per cent), reptiles (6 to

TABLE 6

Gray (brown) rat—Percentage weight of skin, skeleton, musculature, viscera and remainder

SEX	NET BODY WEIGHT	SKIN	SKELETON	MUSCULATURE	VISCERA	REMAINDER
	grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
m.	65.0	18.42	13.15	35.39	23.40	9.64
m.	95.4	19.29	13.85	38.57	23.21	5.08
f.	107.5	20.37	13.86	42.14	17.51	6.12
m.	164.0	17.35	13.29	41.66	20.95	6.75
f.	254.0[1]	19.41	10.16	44.21	16.22	10.00

[1] Including gravid uterus, which weighed 13.76 grams.

21 per cent), amphibia (13 to 21 per cent), and fishes (5 to 13 per cent). These differ so widely in the structure of the integument (especially the appendages), however, that their relative weights are scarcely comparable with each other or with those of mammals. This is also true to a certain extent even for the various mammals. Of mammals, Welcker and Brandt give data for the relative weight of the integument as follows: shrew mouse (Sorex), young, 33.3 per cent, adult, 14 per cent; mouse, 17.6 per cent; bat, 19.5 per cent; mole, 20.2 per cent; hedgehog, 24.3 per cent; guinea pig, 19.8 per cent; monkey, 10.4 per cent; seal, 19.6 per cent; elephant, 13.1 per cent. Sedlmair finds the skin of a well-nourished cat forms 14.7 per cent of the body weight; and Weiske, for rabbits, 14.3 to 16.2 per cent. For dogs (Dachshund), Falck finds the average in 4 newborn 21.8 per cent; at 72 days,

25.4 per cent; 76 days, 18.8 per cent; 108 days, 16.6 per cent; 113 days, 21.3 per cent.

For cattle and sheep numerous data are available. Lawes and Gilbert for 2 fat calves (9 to 10 weeks) find the hide averages 6.9 per cent of the body weight; for 16 adult cattle, 7.5 per cent. Martiny in 3 groups (10 each) of beef cattle found the average 7.8 per cent, 9.1 per cent and 8.0 per cent, respectively. For sheep, Lawes and Gilbert find in a fat lamb (6 months), the integument forms 9.6 per cent (skin proper, 5.9 per cent, wool, 3.7 per cent); in adults, the average of 249 sheep was 11.7 per cent, ranging from 14.1 per cent (in 5 thin yearlings) to 10.5 per cent (45 very fat adults, $1\frac{3}{4}$ years). Henneberg in 19 sheep finds the skin forming 7.1 per cent to 12.5 per cent. Long in 2 fat sows finds the skin (without fat) to form 4.9 per cent and 5.1 per cent.

The foregoing data are reckoned in percentage of the net body weight, excluding contents of the alimentary canal. Voit has pointed out further that if the state of bodily nutrition, the amount of fat and hair, etc., present are taken into account, the variations in percentage weight of the body for the different organs are much less. Thus in 6 dogs, the skin varied from 8 per cent to 19 per cent of the body weight; but reckoned on the fat-free, hair-free basis, the variation was only from 7 per cent to 9 per cent for the well-nourished, and from 7 per cent to 13 per cent for the poorly-nourished animals.

The relative weight of the skin in the rat is thus high as compared with that of most mammals. In general, the skin evidently forms a relatively larger percentage of the body weight in small animals, which is to be expected, since in these the surface area is larger in proportion to the mass of the body.

Skeleton (tables 3, 4, 6; fig. 2). The skeleton (including bones, cartilages and ligaments) like the skin, apparently increases relatively for a short time after birth, the average percentage being 17.27 per cent at birth, and 18.47 per cent at 1 week (table 4, fig. 2). Thereafter it diminishes steadily at the various ages studied, reaching an average of 10.91 per cent at 1 year. The high figure for one of the females at 1 year (15.53 per cent) is probably either an error or an abnormality. There are no constant differences

apparent between the sexes, and the limited data for the gray rat (table 6) are well within the limits of variation for the albino.

Of data available for comparison with other forms, Vierordt estimates the skeleton in the human newborn at 13.7 per cent; in the adult, 17.5 per cent. Mühlmann estimated that the human skeleton increases from 12.6 per cent in the newborn to a maximum of 20.4 per cent at 11 to 20 years, thereafter decreasing to 10.1 per cent in old age.

Welcker and Brandt in addition to numerous data for the skeleton of birds (7 to 13 per cent), reptiles (7 to 43 per cent), amphibia (7 to 10 per cent), and fishes (6 to 17 per cent), give the following for mammals: mouse, 8.4 per cent; bat, 14.6 per cent; hedgehog, 11.1 per cent; guinea pig, 8.8 per cent; seal, 11.1 per cent; monkey, 16.8 per cent; elephant (skeleton plus musculature), 69.7 per cent. Falck for Dachshund, newborn (average of 4), 14.4 per cent; nearly grown, 14.9 per cent; adult, 14.0 per cent. Sedlmair, for well-nourished cat, finds 10.1 per cent; Weiske, for rabbits, 8.1 per cent to 9.2 per cent.

Martiny, in 3 groups (10 each) of beef cattle, finds the skeleton averages 14.3 per cent, 14.6 per cent and 15.3 per cent, respectively. Henneberg gives average of 2 lambs, 10.4 per cent; and of 8 adult sheep, 4.4 per cent to 7.1 per cent, being least in the fattest animals. Long, for 2 fat sows, gives 6.3 per cent and 6.7 per cent.

An inspection of the data given above shows great variation in the relative weight of the skeleton, even in animals close together in size and relationship. This is due partly to differences in skeletal structure and in the amount of musculature (with which the skeletal system is to a certain extent correlated) and partly to variation in the state of bodily nutrition. Voit shows, for instance, that among dogs whose skeleton varied from 12 per cent to 29 per cent of the body weight (as ordinarily reckoned), if reckoned upon a fat-free, hair-free basis the percentage weight varies from 14 per cent to 15 per cent in well-nourished, and from 17 per cent to 30 per cent in poorly-nourished animals.

Musculature (tables 3, 4, 6; fig. 2). Unlike the skin and skeleton, the musculature (including tendons) appears to decrease slightly in relative weight, from an average of 24.37 per cent to

22.82 per cent during the first week (table 4, fig. 2). Thereafter it increases steadily in relative weight, reaching an average of 45.43 per cent at 1 year. No noteworthy differences appear between the sexes, or in the gray rat (table 6).

The musculature forms so large a mass that its growth virtually dominates the body. Thus during the first week, when the musculature lags behind in growth, the other constituents push ahead and increase their relative weights. Later, however, when the musculature assumes its characteristic more rapid rate of growth, it forges ahead and the other constituents necessarily decrease steadily in relative weight up to the adult condition. The increase in the relative proportion of the musculature between the newborn and the adult is also characteristic of man, and perhaps to a slighter extent in other animals. No data are available to show whether there is in any other species a temporary decrease after birth as shown above for the rat.

For the human newborn, Welcker and Brandt cite 2 cases from Bischoff in which the musculature formed 23.3 per cent and 24 per cent of the body. Vierordt estimates for the human newborn, average, 25 per cent, and for the adult, 43.2 per cent. Mühlmann for newborn estimates 22.4 per cent, increasing to 43.2 per cent at 41 to 50 years, thereafter decreasing to 18.6 per cent in old age.

Welcker and Brandt, in addition to numerous birds (36 to 55 per cent), reptiles (19 to 57 per cent), amphibia (43 to 54 per cent), and fishes (49 to 59 per cent), give the following for the musculature of mammals: bat, 41.6 per cent; mouse, 43.4 per cent; hedgehog, 36.6 per cent; guinea pig, 45.8 per cent; monkey, 53.5 per cent; elephant (skeleton plus musculature), 69.7 per cent; ox (skeleton plus musculature), 64 per cent; deducting 15 per cent for skeleton (Martiny) gives 49 per cent. Falck, for Dachshund, finds in newborn, average, 36.2 per cent; nearly grown, 38.4 per cent; adult, 39.6 per cent. Sedlmair, for cat, finds 57.2 per cent; and Weiske, for rabbits, 49.7 to 57.2 per cent. Lawes and Gilbert, in a lean pig, find 41.6 per cent for muscle, and in a fat pig, 30.9 per cent. Long, in 2 fat sows, finds 29.7 per cent and 32.6 per cent. Henneberg, in 2 lambs, finds the average 34.4 per

cent; in 8 adults, 20.3 per cent to 33.2 per cent, the lower percentage corresponding to the fatter animals.

Voit, in the 6 dogs previously mentioned, finds the musculature forming 36 per cent to 49 per cent of the body weight. Reckoned on a fat-free, hair-free basis, however, the variation is only 52 to 55 per cent for well-nourished, and 40 to 48 per cent for poorly-nourished animals.

Thus in comparison with other animals with respect to the relative weight of the musculature, the rat occupies an intermediate position. In most adult mammals, the musculature forms between 40 per cent and 50 per cent of the body weight. Theoretically, as Welcker has noted, a larger relative weight of muscle might be expected in a larger animal. This is because the functional capacity (tension strength) of a muscle varies as the cross-sectional area, which would increase only in proportion to the square of a linear dimension, while the mass of the body to be supported and moved would increase as the cube of the same dimension. The data do not seem to confirm this theory, however. For example, the percentage of muscle is nearly the same in the mouse as in the elephant. Among mammals, the largest percentages of muscle recorded are in comparatively small animals (rabbit, cat), while the smallest relative weights are found in comparatively large animals (pig, sheep).

4. Viscera (tables 3, 4, 6; fig. 2). Like the skin and skeleton, the visceral group (including the central nervous system, thoracic and abdominal viscera) increases in relative weight immediately after birth. In the newborn, it averages 18.05 per cent, increasing to 19.17 per cent at 1 week, and continuing to increase to a maximum of 21.28 per cent average at 3 weeks. Thereafter it diminishes gradually in relative weight, reaching an average of 13.3 per cent at 1 year. There are no evident differences between the sexes. The data for the gray rat (table 6) appear constantly higher than those for the albino of corresponding body weight. Whether this is really a constant difference is somewhat doubtful, however, on account of the small number of observations on the gray rat.

The visceral group in the human newborn averages about 24 per cent of the body weight (Jackson). According to Vierordt, it forms 23.4 per cent in the newborn, decreasing to 9.8 per cent in the adult. Accurate data for comparison of the intermediate stages are not available for man or for any other animal. In the pig, Lowrey finds about 16 per cent for the newborn, and 7.8 per cent for the adult. A smaller decrease between newborn and adult (21.8 per cent to 19.5 per cent) is shown by Falck's data for the dog.

Welcker and Brandt, in addition to data for various birds, reptiles, amphibia and fishes, give data from which the following have been calculated for mammals: bat, 19.5 per cent; shrew mouse, young, 21.1 per cent, adult (includes intestinal contents?), 31.7 per cent; mouse, 22.3 per cent; guinea pig, 17.6 per cent; hare, 16.3 per cent; sheep, 11.5 per cent; monkey, 12.8 per cent; ox, 10.3 per cent; elephant, 12.5 per cent. Sedlmair, for cat, finds 14.5 per cent; and Baumeister for adult pig, in medium condition, 6.0 per cent, fat, 9.8 per cent.

Voit, in the 6 dogs before mentioned, finds the visceral group forming 17 per cent to 22 per cent of the body. (Viscera in this case include blood, but not heart). When calculated on the fat-free, hair-free basis, however, unlike what was found for skin, skeleton and musculature, the variation scarcely appears less. In well-nourished animals, it was found to be 19 per cent to 22 per cent, and in the poorly-nourished, 17 per cent to 25 per cent.

In general, the smaller mammals have a relatively larger visceral apparatus, probably correlated with a more intense metabolism. The rat occupies a somewhat intermediate position, the relative weight of the viscera being less than that of most of the small mammals, but greater than that of the larger mammals.

5. Remainder (tables 3, 4, 6; fig. 2). The remainder is the amount obtained by subtracting from the net body weight the weight of the skin, skeleton, musculature and visceral group. In addition to the liquids escaping from the tissues and body cavities and the loss by evaporation, it includes a few small unweighed organs (genitalia, aside from gonads, larynx, trachea,

pharynx, oesophagus, large vessels) and varying amounts of fat in connection with the muscles and abdominal cavity.

It will be noted (table 4, fig. 2) that in the newborn the remainder forms a considerable proportion of the body (average 20.56 per cent). It decreases, at first very rapidly, reaching an average of 13.68 per cent at 1 week, and then more slowly to 12.85 per cent at 3 weeks. From this time onward, it remains on the average rather constant, between 12 per cent and 13 per cent, but with considerable individual variation, due chiefly to the varying amounts of fat present. There is no evident variation according to sex; but in the gray rat the remainder appears low (table 6), probably because there is usually less fat present.

The remarkable decrease in the remainder during the first week naturally calls for an explanation. It cannot be due to varying amounts of fat, for no appreciable amount is visible at that age. There is likewise no considerable variation in the small organs included in the remainder. The decrease is apparently to be explained as follows. The newborn rat is very "juicy," or rich in water, with relatively large amounts of liquid in the interstitial tissue spaces as well as in the various cavities of the body. This excess of liquid largely disappears during the first week, and the remainder is thereby very markedly diminished.

In the human newborn, the remainder is apparently not more than 15 per cent. Vierordt's data give a remainder of about 18.1 per cent for the newborn, and 11.6 per cent for the adult. This, however, includes the intestinal contents. There is evidently a decline somewhere between the newborn and the adult, but data upon the intermediate stages are lacking. In the dog, as shown by Falck's data, the remainder is apparently about the same in the newborn (5.8 per cent) as in the adult (5.7 per cent). In a well-nourished cat, the remainder forms 3.5 per cent (Sedlmair). From data by Welcker and Brandt, remainders were calculated as follows: ox, 17.6 per cent; sheep, 14.5 per cent; mouse, 10.3 per cent; guinea pig, 7.9 per cent; hare, 7.2 per cent; monkey, 6.5 per cent; elephant, 4.8 per cent. The high proportion in the ox and sheep is due to their excess of body fat, and, as in the case

of the rat, variations in the adults of other animals are apparently due chiefly to difference in this respect.

In regard to the relative size of the various systems, as already mentioned for the component parts, practically the adult relations have been reached at the age of 10 weeks. This is evident from figure 2, but would appear still more striking if the horizontal distance beyond 10 weeks were increased to scale in proportion to the length of time up to 1 year. The only change of note after 10 weeks is the slight relative increase in the musculature, which is balanced by a decrease in the other systems, chiefly in the viscera.

CONCLUSIONS

The more important conclusions may be summarized briefly as follows:

1. The head of the albino rat increases in relative weight from an average of about 23 per cent of the body in the newborn to nearly 26 per cent at 1 week. Thereafter it decreases in relative size, forming about 10 per cent of the body in the adult rat (age, 1 year).

2. The upper extremities increase from about 7 per cent of the body in the newborn rat to about 9 per cent at 3 weeks, thereafter decreasing to less than 5 per cent at 1 year. The lower extremities increase steadily from about 9 per cent at birth to about 15 per cent at 1 year.

3. The trunk decreases from an average of about 60 per cent of the body at birth to about 52 per cent at 3 weeks, increasing thereafter to about 70 per cent at 1 year.

4. The skin increases rapidly from about 20 per cent of the body in the newborn to nearly 26 per cent at 1 week; thereafter it decreases steadily to about 18 per cent at 1 year.

5. The skeleton increases slightly from an average of about 17 per cent of the body in the newborn to about 18 per cent at 1 week; thereafter it decreases steadily in relative weight, to about 10 per cent in the adult.

6. The musculature decreases relatively from an average of 24.4 per cent of the body in the newborn to 22.8 per cent at 1

week; thereafter it increases steadily, averaging slightly more than 45 per cent of the body in the adult rat.

7. The visceral group increases from an average of 18 per cent of the body in the newborn to about 21 per cent at 3 weeks; thereafter decreasing to nearly 13 per cent at 1 year.

8. The remainder (net body weight minus skin, skeleton, musculature and viscera) undergoes a striking decrease from an average of about 21 per cent of the body at birth to about 14 per cent at 1 week. This is due to the disappearance of excessive liquids in the newborn. After 3 weeks, the remainder averages slightly more than 12 per cent of the body.

9. The body of the albino rat has practically reached the adult proportions in its component parts and systems at the age of 10 weeks.

10. The data indicate no noteworthy differences between the sexes in the relative weight of the various parts and systems. With the possible exception of slightly heavier viscera and smaller remainder, the few data on the gray rat likewise reveal no marked difference in this form.

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DUPLICATION OF THE INFERIOR VENA CAVA IN MAN

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TWO FIGURES

The two cases of duplication of the inferior vena cava described below were found in the dissecting room of the Cornell University Medical College at Ithaca.

The first case occurred in a colored male, aged forty-seven, who died of general paresis, cadaver no. 466 of the Cornell series.

The common iliac veins of both sides are formed in the usual way by the junction of the external and internal iliacs. The right common iliac vein after a course of 4.5 cm. is joined not by the whole of the left common iliac but only by a large branch from it, the ramus communicans. The right inferior vena cava thus formed extends for a distance of 11 cm. as a large independent stem and is then joined by the left inferior vena cava and then forms the common vena cava and runs superiorly as a single trunk for 2.5 cm. when it enters the fossa for the vena cava in the liver and continues to the heart. The right common iliac vein begins at the level of the anterior superior iliac spine. It receives the ramus communicans at the superior border of the 5th lumbar vertebra and is joined by the left inferior vena cava at the inferior border of the first lumbar vertebra.

The left common iliac vein runs superiorly for a distance of 2 cm. when it divides into two branches of about equal size. The one mentioned above as the ramus communicans passes obliquely across the body of the 5th lumbar vertebra to join the right inferior vena cava. The other branch which represents

the left inferior vena cava continues superiorly along the spinal column lateral to the aorta and nearly parallel to the right inferior vena cava. At the inferior border of the first lumbar vertebra where the lumbar veins enter it bends abruptly to the right and passes ventral to the aorta to join the inferior vena cava. The transverse part of it corresponds in position with the renal vein in a normal case.

An idea of the relative size of the different vessels is obtained from the following measurements which, in all cases, were made upon empty flattened vessels since all the vessels could not be fully distended:

Right inferior vena cava	3.0 cm.
Left inferior vena cava	1.6 "
Right common iliac vein	2.5 "
Left common iliac vein	2.0 "
Ramus communicans	1.7 "

The right inferior vena cava opposite the point where the left joins it receives three renal veins of 1.2, 0.6, and 0.7 cm. in diameter, the superior being the largest, and receiving the right suprarenal vein. The right inferior vena cava receives four lumbar veins.

The left inferior vena cava opposite its junction with the right receives five separate veins. Of these the most superior is the left suprarenal which is about twice as large as the right suprarenal. Three receive blood from the kidney. The most ventral is the largest renal vein, being 1.2 cm. in diameter, while the other two renal veins are over 0.3 and 0.6 cm. in diameter. The most posterior, a vein 0.6 cm. in diameter, is the left ascending lumbar vein. Its continuation is the hemiazygos (vena azygos minor). It receives a small anastomosing branch from the azygos vein (vena azygos major) 3 cm. before its junction with the left inferior vena cava. The left inferior vena cava receives, in addition to the ascending, two other lumbar veins, one 2 cm. inferior to the left ascending lumbar vein and the other 1 cm. superior to the beginning of the ramus communicans.

The right spermatic vein empties at the junction of the right and left inferior venae cavae. The left spermatic vein joins the

left inferior vena cava; 5 cm. inferior to the entrance of the large anterior renal vein (fig. 1).

In the fall of 1911 a condition similar to the first was found in another subject (no. 401, Cornell series), a white male, aged fifty, who died of cirrhosis of the liver.

On the right the external iliac vein is joined at the level of the anterior superior iliac spine by the internal iliac vein to form the

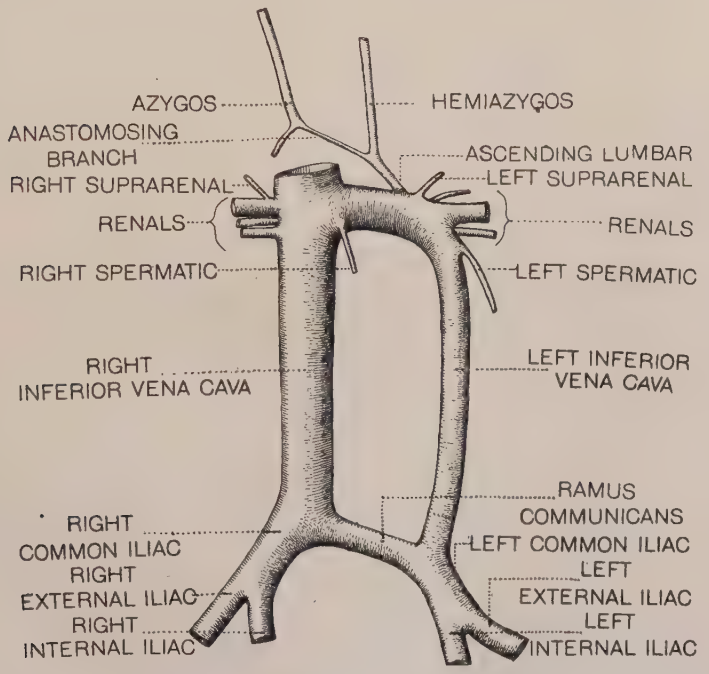

Fig. 1 Semi-diagrammatic drawing of the venae cavae of subject no. 466.

right common iliac vein. The right common iliac vein then extends superiorly to a level corresponding to a plane, passing through the lower quarter of the right kidney, where it is joined by the left common iliac vein to form the vena cava. From this point the inferior vena cava extends superiorly for a distance of 2.5 cm. It then receives two large renal veins: one from each kidney, and extends in the usual way to the heart.

The left internal and external iliac veins join at a level slightly lower than the right to form the left common iliac vein. This is joined 4 cm. from its origin by a small branch which connects it with the right internal iliac vein and it then extends 10 cm. farther superiorly to join the right as already noted.

On the left side it is quite evident that the vessel superior to the branch which connects with the right side should be considered a left inferior vena cava and it is worthy of note that it is a larger vessel than the corresponding vessel on the other side. On the right side it is not quite so clear where we should consider the inferior vena cava as beginning since the ramus communicans connects with the right internal iliac and not as is usually the case with the common iliac vein. This branch is quite small, measuring 2 or 3 mm. in diameter when flattened out. It receives the middle sacral vein.

The left common iliac vein, 2 cm. before its junction with the right, receives the right spermatic vein. The left renal vein receives the left spermatic vein.

There are a number of loops formed in the veins. A large branch joins the right external iliac vein, the right internal iliac vein forming a loop which encloses the external iliac artery. This loop receives the right superior gluteal vein and another small vein. The right common iliac vein about the middle of its course gives off a short branch which runs for 1.5 cm. and again joins the right common iliac vein forming a loop which receives a small lumbar vein (not shown in the figure).

The left external iliac vein gives off a small branch which joins the left internal iliac vein about 2 cm. before their junction. This loop is smaller than that of the other side and the artery does not pass through it. There is, however, another loop formed by the left internal iliac vein opposite the point where the left superior gluteal vein joins it. This encloses the left internal iliac artery.

I have been able to find reports of but 14 similar cases and for comparison will give a brief résumé of them. They have been divided into two groups; those in which the left inferior vena cava is connected by anastomoses with the right and those in which it is not.

Cases with no anastomoses between the two cavae

In Kollman's case in an adult, the right vena cava was 15 cm. long from junction of external and internal iliac and received the right renal vein: while the left vena cava was 17 cm. long and received the left renal vein. The two venae cavae joined to form a single vein which connected with the heart, and which was 10 cm. long from heart to the level of the superior mesenteric artery.

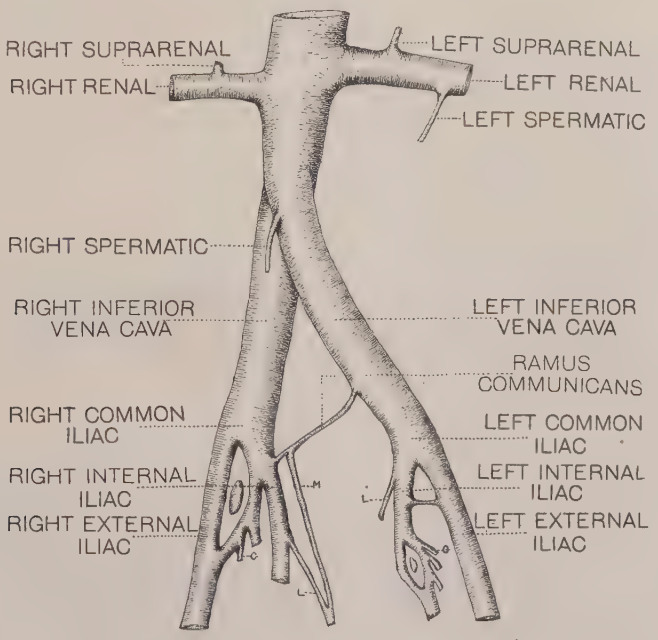

Fig. 2 Semi-diagrammatic drawing of the venae cavae of subject no. 401. *M*, middle sacral vein; *L*, lateral sacral vein; *G*, superior gluteal vein.

Zaaijer reports a case in a male sixty-two years old in which the inferior vena cava was normal but there was a branch parallel to the aorta establishing a communication between the left common iliac vein and left renal vein. This undoubtedly should be considered a left inferior vena cava. It is interesting to note that there was no right kidney in this case.

Flesch describes a case in which the left internal iliac vein passes superiorly along the aorta to join the left renal vein forming with this a

single vessel which crossed the aorta at the level of the second lumbar vertebra to join the right inferior vena cava. The azygos and hemiazygos veins remained normal.

In one of Nicolai's cases there was no anastomosis between the two cavae and each received tributaries from the corresponding side of the body. The right vena cava was normal to the lower half of the liver, where it joined with the vena cava from the left side. The left vena cava arose at the same level as the right and passed superiorly on the left of the aorta to the level of the hilus of the kidney, where it received the left renal vein and then ran over to the right to join the right vena cava opposite the point where this latter received the right renal vein. Each inferior vena cava was 5 cm. in diameter at the point of junction. The left suprarenal vein opened into this oblique portion of the left renal vein. The common trunk was 4.5 cm. long, and 2.5 cm. broad. Each of the separate inferior cavae received a spermatic and also lumbar veins.

Broca very briefly describes a case by Zagorsky in which the two primitive common iliac veins joined at the level of the articulation of the 1st and 2d lumbar vertebrae. Each of these received its corresponding renal, spermatic and lumbar veins, hence we must conclude that it is a case of double inferior vena cava.

Cases with anastomoses between the two venae cavae

In Wilde's case, an adult, the right vena cava (called by him 'common iliac') joined near the liver with the left which passed over the aorta. Each of the cavae received four lumbar veins, and a spermatic vein. The right received one and the left two renal veins; the left vena cava received the left suprarenal and the unpaired stem the right suprarenal. At the level of the 5th lumbar vertebra the two cavae are joined together by an anastomosis running behind the aorta obliquely from the left upwards to the right.

Nicolai found in a 74-year-old woman the right inferior vena cava 1.4 cm. broad formed by the junction of the right common iliac vein with a branch from the left of ramus communicans (called by him the 'left common iliac'). From its formation at the level of the bifurcation of the aorta the right cava ran at the right of the aorta a distance of 9 cm. to the level of the hilus of the kidney where it was joined by the left inferior vena cava. The two cavae here formed a common stem 2 cm. broad. At the point where the internal and external iliac veins

join, they divide into the ramus communicans and left inferior vena cava. This latter ran vertically for 11 cm. to the height of the hilus of the kidney where after a horizontal course of 5.5 cm. it joined the right vena cava. There were two right renal veins, both entering the right vena cava opposite the junction of the left. The superior of these received the vena azygos. On the right a spermatic vein could not be seen and but two small veins that could be called lumbar veins. The common stem received only the right suprarenal vein which is very short. In its inferior part the left vena cava received the left spermatic vein. Where the left vena cava bent to join the right it received the left renal vein. The horizontal portion of the left vena cava received a large vein which was formed by a short suprarenal and the vena hemiazygos.

In Lobstein's case the common vena cava at the accustomed entering place of the renal veins received the right and left venae cavae. The common stem received the right renal, right suprarenal, and a small stem that helped to form the vena azygos. The right vena cava received the right spermatic vein. The left vena cava received the left suprarenal, renal, and spermatic veins. No mention is made of lumbar veins. A strong anastomosis occurred between the venae cavae running from the place of union of left external and internal iliac veins (which union lies somewhat deeper than the right), obliquely superiorly to join the right vena cava. The middle sacral vein came off from this anastomosing branch.

Lagneau found in a young man that the left primitive iliac vein ascended parallel to the aorta to form the left inferior vena cava which joined the right, at the level of the kidneys. In its superior part it received: the left testicular vein, a large trunk resulting from the union of two large left emulgent veins, and a capsular vein of the same side. The right external and internal iliac veins formed the 'primitive iliac vein,' the ascending vein of the right side. Similarly, the left external and internal iliac veins communicating through a vast anastomosis formed the 'primitive iliac' and as such arose as the left inferior vena cava. The left external iliac vein received behind the primitive iliac artery a considerable trunk resulting from the union of the two internal iliac veins.

Besides the two inferior venae cavae, one can observe on this subject first, that the two internal iliac veins and the left external iliac vein joined in order to form the trunk of the left inferior vena cava, while the right inferior vena cava received only the right external iliac. Sec-

ond, that on each side an anastomosis existed between the external and internal iliac veins.

Le Gendre found in a foetus with enlarged pelvis of kidney the common iliac veins continued superiorly as two venae cavae. The left vena cava crossed the aorta obliquely to join the right at the level of the upper pole of the kidney. The right vena cava received the right renal vein while the left vena cava received two renal veins superior to the level of the ramus communicans. At the level of the hilus of the kidney the ramus communicans passed behind the aorta to connect the two cavae. No further description of the venous system is given.

Gruber describes a case in a man in which the common iliac vein ascended on both sides of the aorta as a double vena cava to join at the level of the first lumbar vertebra. The aorta 4 cm. wide running obliquely inferiorly for 12 cm. was surrounded by a 'verschobenen parallelagrammatischen' ring formed by the common iliac veins and their anastomoses. The right vena cava and its stems ran normally, only the vein was a little farther laterally from the aorta than usual. The left common iliac vein lay at first behind the left internal iliac artery and the lower part of the left common iliac artery bent behind these, and ascended on the left of the aorta to the second lumbar vertebra and then crossed obliquely over the aorta at the lower border of the mouth of the superior mesenteric artery to join the right vena cava at the level of the first lumbar vertebra. There was a ramus communicans running from the left obliquely superiorly behind both stems of the aorta to join the right vena cava at the level of the 4th lumbar vertebra. The vena sacralis media was received by the ramus communicans. The right vena cava received the right renal, spermatic and four lumbar veins. The left vena cava received the left suprarenal, left renal, spermatic, and four lumbar veins. The azygos and hemiazygos veins were normal.

Le Gendre describes in another man a case somewhat similar. The common iliac veins ran up on both sides of the aorta to join in front of the intervertebral fibro-cartilage between the last thoracic and first lumbar vertebra. Both cavae, being of about the same calibre, were connected by a ramus communicans which comes off partly from the left vena cava and partly from the left internal iliac vein and joins the right vena cava about 1 cm. above the junction of external and internal iliac veins. The ramus communicans received the vena sacralis media

in its right half. The left vena cava ascended vertically to the upper border of the second lumbar vertebra and then crossed obliquely to join the right. The unpaired vena cava thus formed received the right suprarenal vein. The right renal, right lumbar, and the right spermatic vein emptied into the right vena cava especially low. The left vena cava received the left suprarenal, renal, spermatic, and four lumbar veins. The vena azygos and hemiazygos are normal.

Walter described a case in a man in which the two venae cavae joined at the hilus of the kidney to form a short common vena cava inferior. The right vena cava ran vertically on the right of the aorta and received the two right renal, the right spermatic, and the four right lumbar veins. The left vena cava ran vertically on the left of the aorta and received the left suprarenal, renal, spermatic, and lumbar veins. A ramus communicans of about the same calibre as the vena cava running obliquely superiorly from right to left connected the two at the level of the 5th lumbar vertebra.

Walter described a second case in a woman in which two large venous stems joined at the level of the 1st lumbar vertebra to form a common inferior vena cava. The left stem ascended vertically to the point where it crossed the aorta obliquely to join the right vena cava which ascended on the right of the aorta. On each side the venae cavae arose in their normal place from the internal and external iliac veins. The right iliac vein lay normally while the left ran lateral to its artery. From the right common and partly from the internal iliac veins at the junction of internal and external iliacs the reasonably large ramus communicans arose. It ran across the vertebral column between the 4th and 5th lumbar vertebrae obliquely upward to join the left common iliac. In front of the second sacral vertebra there was a short anastomosis between the internal iliacs. At the level of the 4th lumbar vertebra there was a small ramus communicans running behind the aorta to connect the two venae cavae. The unpaired vena cava received the right renal vein. No mention is made of any further tributaries of the venae cavae.

In the foregoing cases the two cavae joined one another between the limits of the 12th thoracic and the 2d lumbar vertebra. In those cases having a ramus communicans the two cavae joined between the 1st and 2d lumbar vertebra while in the others the point of junction was of more variable extent. The cavae are of variable lengths, but in the majority of cases the left was longer than the right as we would naturally

expect. Sometimes the ramus communicans ran behind the aorta but usually in front of it. The superior connection of the two cavae corresponding to the left renal vein did not pass behind the aorta in any case.

The inferior vena cava is now considered to be a compound vessel consisting of a part of the heart, part of the vena hepatica communis, dilated sinusoids of the liver, part of the right subcardinal vein, and a section of the right posterior cardinal vein.

As has been shown by various investigators, more recently by Lewis in the rabbit embryo, the iliac veins empty into the post cardinal vein on each side. Running parallel anteriorly with each of these posterior cardinal veins there is later developed another vein, the subcardinal. These veins have numerous anastomoses with the posterior cardinal and with one another. At the level of the future left common iliac, a strong anastomosis develops between the two posterior cardinals. A second strong anastomosis develops at the level of the future renal veins. This connects the posterior cardinal and subcardinal veins on the two sides. Both of these anastomoses persist and later become more strongly developed. On the left side the posterior cardinal and subcardinal between these crossed anastomoses normally disappear. The most inferior crossed anastomosis enlarges and becomes the left common iliac vein. The superior transverse anastomosis persists and enlarges to form the right connection between the persistent posterior and subcardinal veins. The connection with the right posterior cardinal superior to this point is lost and the right subcardinal inferior to the connecting branches disappears or becomes very small. On the left side the anastomosing branch also enlarges and becomes the renal.

If the above interpretation of the developmental changes is correct, we have in the first specimen described by me a persistence, instead of a disappearance, of the left posterior cardinal and possibly also in the neighborhood of the renal, of the subcardinal. The blood instead of all passing through the left common iliac, as is normal, divides and part passes through the persistent posterior and subcardinal veins as far as the superior crossed anastomosis where joining with the blood from the renal

and suprarenal it crosses in the persistent superior crossed anastomosis to empty into the right vena cava. In the second specimen in place of the left posterior cardinal remaining persistent as far as the superior crossed anastomosis (renal) one of the numerous secondary anastomoses which connect the posterior and subcardinal inferior to this level has become enlarged and remains as the permanent connection between the right and left sides. In addition to this the inferior transverse anastomosis which usually becomes the left common iliac vein has become very much reduced and persists only as a very small connecting vein. This specimen shows a persistence of several loops so frequently seen in developing veins but as a rule disappearing in this vein.

The majority of the cases described in the literature correspond very closely with the first case of mine. In those specimens described by others in which there was no inferior connection between the two sides, the crossed anastomosis which normally develops into the left common iliac vein either completely disappears or did not develop at all.

I wish to thank Professor Kerr for his valuable suggestions and criticisms.

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THE RELATION OF THE SINO-AURICULAR NODE TO THE VENOUS VALVES IN THE HUMAN HEART

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ONE FIGURE

The reason for publishing this finding in the human heart is to present evidence that the sino-auricular node lies within the region which corresponds to the sinus venosus of the cold-blooded vertebrates. The sino-auricular node is now believed by many to be the site of origin of the heart beat in mammals,[1] just as the sinus venosus is known to be the primum movens in the cold-blooded vertebrates. In the lower vertebrate heart, the sinus venosus is a distinct chamber separated from the auricular canal by the venous valves, so that the free margin of the valves forms the boundary line between the cavity of the sinus and that of the auricular canal. But as in the adult human heart the venous valves in this region have been greatly modified and shifted in position, it has not been definitely shown on which side of the valve the sino-auricular node is located.

In the hearts of the two foetuses and the one child's heart about to be described we found the venous valve present and the sino-auricular node (that is the pacemaker of the heart) to the sinus side of the valve. The hearts, which were secured through the courtesy of Dr. E. A. Park and Dr. B. Rosenbluth, were from an infant three weeks old which had died of pneumonia and from two foetuses 16 cm. and 21 cm. long, respectively.

Upon gross examination the infant's heart was found to be normal. The heart was fixed in alkalinized formalin. For microscopic exami-

[1] See the work of Wybauw, Lewis and Oppenheimer, Cohn and Kessel, Brandenburg and Hoffmann, Ganter and Zahn, and the opinions of W. Koch and of Hering.

nation the region of the sino-auricular node was excised. This block consisted of the wall of the right auricle on either side of the sulcus terminalis of His; extending dorsad as far as, and including a portion of the endocardium of the left auricle and a part of the superior and inferior venae cavae, extending ventrad beyond the crista terminalis of His so as to include a strip of pectinate muscles all along the length of the crista. Moreover this block extended along both sides of the crest of the right auricle, thus including not only the part already described on the lateral aspect of the auricle on either side of the sulcus, but also the auricular wall on the median aspect facing the aorta. On the lateral aspect the area reached almost to the auriculo-ventricular groove; on the median face it extended from the crest down to the level of the origin of the aorta. This piece was embedded in celloidin-paraffin, cut into sections 15 micra thick, and stained with Weigert's iron-haematoxylin and van Gieson's picric-acid fuchsin solution. The sections were horizontal, that is perpendicular to the length of the crista terminalis of His and to the epi- and endocardium.

On microscopic examination the typical nodal tissue was found in the usual position, namely, at the junction of the superior vena cava with the right auricle and along the crista terminalis under the sulcus of His. The node could be identified even under low magnification by its wealth of connective tissue, crowded nuclei, and the dense syncytial character of its musculature; moreover by its relationship to its nutrient artery.

At the junction of the node and crista a valvular cusp was found, as well as its companion cusp on the opposite side of the endocardium. These cusps consist of a central core of connective tissue with a few muscular elements, covered with a single layer of flattened endocardial cells. The crista and the pectinate muscles were situated anterior to the valves; the musculature of the superior vena cava, atrium and sino-auricular node posterior to the valves; the endocardium covering the auricular wall in front of the valves is thinner than that covering the auricular wall posterior to them.

In Keith and Flack's classic papers,[2] the position of the venous valve in the human heart was suggested in the exact situation in which we have actually found it in the heart here described. Keith and Flack identified the remnant of the sino-auricular junction in the human heart by comparing it with the turtle's

[2] Keith and Flack, Jour. of Anat. and Physiol., 1907, vol. 41, p. 172.

Fig. 1 Showing horizontal section of the right auricle in the region of the sino-auricular node of an infant's heart. Photomicrograph × 16. *1–1*, venous valve; *2*, ordinary cardiac musculature; *3*, musculature of the sino-auricular node; *4*, taenia or crista terminalis of His, which, lined with thin endocardium, lies on the auricular side of the valve. The elongated cavity, lined with thick endocardium, is situated on the sinus side of the valve.

heart in which there is a venous valve. They showed (fig. 6 of their paper) that in the turtle's heart the endocardial covering of the auricular musculature is thinner than that of the sinus, and that the venous valve is situated at the junction of the two varieties of endocardium. In the human heart here described and in the heart of the two foetuses examined, the sino-auricular junction as such is almost as clearly shown as in the more primitive hearts, for the venous valve is actually present lying between the musculature (auricular) covered by a thin endocardium and that (sinus) lined with a thick endocardium. The nodal tissue was found at the base of the venous valve in the region covered by thick endocardium. The relations in the foetuses' hearts were found to be the same as those in the infant's.

In brief, in the infant and foetus' hearts here presented the sino-auricular node lay in close proximity to the base of the venous valve, in what corresponds to the sinus venosus of cold-blooded vertebrates.

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In a recent paper[1] I described the development of the pulmonary arteries in rabbit embryos, and showed that, contrary to the heretofore accepted views, originated by Rathke and His, these vessels are not branches of the last or pulmonary aortic arches, but caudal prolongations of the ventral aortae. These ventral aortae are represented by a network of capillary vessels which grows caudally from the truncus arteriosus, between the ventral wall of the pharynx and the dorsal wall of the pericardial cavity, extending from side to side across the median line, giving off, between the gill pouches, lateral sprouts which join others from the dorsal aortae to form the second, third and fourth aortic arches. To quote from the paper cited:

> A further extension of the plexus of the ventral aorta, situated between the floor of the pharynx and the dorsal wall of the pericardial cavity, but prevented from crossing the median line by the presence of the median pharyngeal outgrowth to form the trachea, reaches to the lungs as the pulmonary arteries, which are later joined by vessels springing from the dorsal aortae. These vessels, which may be double and plexiform, constitute the fifth (and sixth) arches. . . . . The sprouts for this last arch arise chiefly from the dorsal vessels, instead of from the ventral net. I also wish to point out that the net grows beyond the arch, before the arch has become complete. In other words this extension of the ventral aortic net forms well defined pulmonary arteries, one on each side, before the pulmonary arch exists; the pulmonary artery is in no sense a branch of the pulmonary arch, and moreover, in the strictest sense, the arch extends only from the dorsal aorta to the pulmonary artery, the ventral part of the vessel usually called the arch is really the ventral aorta. The persistent pulmonary arteries are entirely ventral; they have been joined during embryonic life by branches from the dorsal aorta, but such branches are only temporary.

On receipt of a reprint of this paper, Dr. V. Fedorow, of the Military Medical Academy, St. Petersburg, Russia, sent me a

[1] Am. Jour. Anat., vol. 13, no. 2, 1912.

copy of a short article[2] published by him in Russian, in which he shows that in the guinea pig the pulmonary arteries arise in the same manner as I have described for the rabbit. Fedorow's work appeared before mine, and I wish to take this opportunity of ascribing to him the priority, and also of bringing to the notice of American anatomists a paper which might well remain unknown because of the language in which it is printed. With this in view I offer the following passages from this article:

> The pulmonary arteries of the guinea pig in the early stages are extremely delicate and can be traced in their surrounding thick mesenchyma only with great difficulty.
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> In the embryo of the eighteenth day, with 30 somites, the first two pairs of aortic arches are obliterated, the third and fourth pairs are fully formed. From the medial wall of the last arches the aa. pulmonales issue on each side. They begin near the truncus arteriosus ventral to the middle part of the pharynx, which is quite large at this point. The arteries run caudally on the ventral surface of the oesophagus, dorsal to the pericardial cavity, and quite near to each other. Further down, the oesophagus appears compressed laterally, and the arteries lie lateral to its ventral part, which projects in the form of a pointed keel. The pulmonary arteries end blindly, traversing about 20 segments (240 m.).
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> In another embryo of the same age, with 29 somites, the arteries extend further, their diameter varying at different points, and it may be anastomose with the vessels of the oesophagus. Similar anastomoses frequently occur later. One notices the double origin of both arteries from their corresponding arches, and they may even join one another near the arch. Island formation occurs along the course of the arteries.
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> In the embryo of the nineteenth day, with 32 somites, the third and fourth pairs of aortic arches are present, the sixth pair represented by blind growths both from the dorsal aortae and from the truncus arteriosus. The delicate pulmonary arteries begin from the sixth pair as short growths. Properly, they are the elongated aa. pulmonales of the earlier stages. They run caudally ventro-lateral to the oesophagus, and anastomose with its vessels.

It will be seen that the two articles run closely parallel to each other; that, though Fedorow speaks of the pulmonary arteries as arising from the fourth arch, while I prefer to call them extensions of the ventral aorta, both agree that they are not originally sprouts from the sixth or pulmonary arches, as has so

[2] Communications of the Military Med. Acad., St. Petersburg, Russia, vol. 22, no. 1, 1911.

long been held true. In two species, rabbit and guinea pig, this has now been worked out. This is the more interesting to me because of the fact that in the further development of these arteries these same two species differ markedly, as pointed out in previous communications.[3] In the rabbit and many other species, including man, the pulmonary aorta becomes the permanent arteria pulmonalis communis, from which the right and left arteries branch, while in the pig and the guinea pig, by an anastomosis of the two pulmonary arteries and the obliteration of one side of the loop thus made (by the proximal portions of the two sixth arches and the proximal portions of the two pulmonary arteries), the permanent arteria pulmonalis communis is much longer, and comprises, besides the pulmonary aorta, the proximal, or ventral, part of the sixth arch (which is really ventral aorta), the upper part of the pulmonary artery of one side, and the anastomosis.

In the pig the left side of the loop remains permanently in communication with the two pulmonary arteries; in the guinea pig the left side becomes obliterated, the right side remains in the adult. In this connection it is of interest that Fedorow, while confirming the facts just referred to, reports in this same paper one case, in a guinea pig embryo of twenty-four days, in which the left side of the loop, instead of the right, remained as a permanent vessel; in other words, in which the guinea pig embryo simulated the pig. This, it seems to me, must be an anomaly, and one that I am unable to explain; any more than I can explain why these two species should differ in this respect as they normally do.

[3] Am. Jour. Anat., vol. 1, no. 2, 1902. Anat. Rec., vol. 3, no. 6, 1909.

BOOKS RECEIVED

THE TERATOLOGY OF FISHES, James F. Gemmill, lecturer in embryology, Glasgow University, and in zoology, Glasgow Provincial Training College, 25 plates, 73 pages including index, 1912. James Maclehose and Sons, Glasgow.

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